Vol. 9, Nos. 7-9 **July-September, 1933** **Nos. 106-108**

Bulletin of the Agricultural Chemical Society of Japan.

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Published by the
Agricultural Chemical Society of Japan
c/o Faculty of Agriculture, Tokyo Imperial University.

Single Copy (Postage inclusive) :- ¥ 0.35
Annual Subscription (12 numbers) :- ¥ 3.50

The Agricultural Chemical Society of Japan.

President : Kintaro OHSHIMA.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Kintaro OHSHIMA.
Associate Editors : Kakuji GOTŌ and Yoshikazu SAHASHI.

On The Natural Pigments of Row Silk Fibre of The Domestic Cocoon (Part VI).

Fading of the Yellow Cocoon.

By

Masami Oku

(From the Chemical Laboratoıy of Gunze Raw Silk Mfg. Co. Ltd., Ayabe-mati, Kyôto-hu, Japan.)

(Received March 27, 1933.)

Yellow cocoons are faded by the drying process and during their storage. They have, on the other hand, characteristic stimulant odor, so-calld "yellow cocoons' odor". The cause of these facts was searched and verified it as resulting from the oxidation of xanthophyll, which is the main yellow pigment of the yellow cocoons.

Experimental.

(1) Oxidation of xanthophyll.

When lutein ($C_{40}H_{56}O_2$) (m. p. 193°) of the yellow cocoon was exposd to the current of oxygen, its yellow tinge was faded and increased in weight gradually, combining with oxygen atom. After 90 days exposure, it was increased in weight by 35% and a composition of $C_{40}H_{56}O_{15}$, melting near about 90°. This oxidized lutein showed no more absorption spectrum as is characteristic to xanthophyll.

(2) Fading during storage.

Yellow cocoons showed no remarkable fading during storage in carbon dioxide atmosphere even after 150 days, while these showed as much as 13 % fading in oxygen atmosphere after the elapse of the same days.

(3) Fading by drying process.

When fresh yellow cocoons were dried for 4 hours at 70°, they lost the yellow tinge as much as 13%.

(4) Presence of xanthophyll on the way of oxidation.

The natural yellow pigments present in the mother liquor of crystalline lutein showed the average composition of $C_{40}H_{56}O_6 \sim O_7$, which deemed me the substance was xanthophyll on the way of oxidation.

(5) "Yellow cocoons' odor".

Xanthophylls both on the way of oxidation and after being utterly oxidized have a violet-like odor, peculiar to the yellow cocoon. Depression of the melting point and increase in volatility by the oxidation of xanthophyll elucidate its smelling power greatly.

On the Chemical Constituents of Rice-embryo.

By

Riang-Ha KIMM and (the late) Taro NOGUCHI.

(Received March 1, 1933.)

Rice-embryo constitutes only 3% by weight of the whole grain and it is very difficult to separate from it in a pure state in large quantities, so its chemical composition has not yet been studied thoroughly.

In 1912, Luigi Bernardini(1) reported on the analysis of the ash and the distribution of phosphoric acid in different forms in the embryo and he pointed out that the embryo is very rich in phosphoric acid of which the greater part is in the phytin-form.

M. Hamada(2) isolated protein from the embryo, determined the distribution of amino acids in its hydrolytic products and demonstrated by feeding experiment that the protein in the embryo has a very high nutritive value. Later, Hirai(3) investigated the ethersoluble substances of the embryo and isolated palmitic, oleic and linolic acids as the chief constituents of fats. Further, from the unsaponifiable fraction, he obtained the so-called Burian's phytosterol, besides a mixture of sterols which was readily soluble in organic solvents. From its high melting point (144°C) and low rotatory power (−23°) it was assumed by him to contain a dihydrositosterol.

Quite recently, the rice-polishing factories, Munechika and Niko & Co. have independently invented new polishing machines by which the embryo can be easily separated from the grain in an intact state and large quantities of these samples were kindly supplied by these factories to the present authors for investigation.

These samples, when carefully refined, looked quite homogeneous and were absolutely free from crushed grains, brans or other impurities. They contained in average 24% fatty matter, including about 1.2% unsaponifiable substances, besides a little waxes and a phytosterine-glucoside (phytosterolin). The waxes chiefly consisted of melissyl cerotate. Of the fatty acids, 25% were saturated and 75% unsaturated acids. From the saturated acids, palmitic, arachidic, and cerotic acids were isolated and identified; further, the presence of a little myristic and stearic acids were proved. The unsaturated acids consisted almost exclusively of oleic and linolic acids. The absence of other higher unsaturated acids was confirmed by the examination of the bromination products.

The unsaponifiable matter was treated with aceton and absolute alcohol and separated into two parts, i. e. a white amorphous powder (80%) and a brown viscous syrup (20%). The former gave strong Liebermann reaction for sterine and the characteristic reaction for ergosterine. By repeated recrystallization from various organic solvents, some melissyl alcohol, a small amount of dihydrositosterine, stigmasterine and the so-called Anderson's γ-sitosterine⁽⁴⁾ were isolated and identified. The existence of a little ergosterine was confirmed by converting it into vitamin D by irradiation with ultraviolet rays. Futher, the mother liquor of the above substances was fractionally crystallized from hexane and a new sitosterine (m. p. 156°C, $[\alpha]_D = -14.49°$) was isolated. It was converted into an acetate (m. p. 111°C, $[\alpha]_D = -9.68°$) and a benzoate (m. p. 129°C, $[\alpha]_D = +14.48°$). This new sterine is comparatively soluble in light petroleum ether and more readily in hexane and other organic solvents. The so-called Anderson's α-sitosterine⁽⁴⁾, which was presumed by him to exist in rice polishing oil, is most probably the mixture of various sterines.

A cerebroside-like substance was also detected in the ether extract of the embryo. Probably it is the same as that obtained by W. Trier⁽⁵⁾ from rice grain. The nature of this substance will be investigated later on.

The authors have thus confirmed that the composition of the embryo oil closely resembles that of the polishing oil,

Experimental.

(I) *General composition of rice-embryo.*

The composition of the purified embryo, supplied from tne Niko & Co., is compared with that reported by each of Sawamura and Hamada in the following table:

	Present authors	Sawamura	Hamada
Moisture	9.83	5.73	10.41
Crude protein	21.26	24.30	20.76
Protein	18.10	——	17.56
Crude fat	23.87	21.05	20.66
Fibre	3.48	9.77	10.11
N-free extract	33.76	25.68	27.82
Ash	9.09	13.47	10.24
Pentosan	5.—	——	——
Carbohydrate	21.—	13.—	——

The composition of the ash is compared with that of Bernardini⁽¹⁾ as follows:

	Present authors	Bernardini		Present authors	Bernardini
P_2O_5	5.020	6.200	MgO	1.104	1.389
SiO_2	0.370	0.250	Mn_3O_4	0.012	trace
Fe_2O_3	0.064	0.060	K_2O	2.022	1.691
CaO	0.312	0.279	Na_2O	0.196	trace

Bernardini moreover determined the distribution of phosphoric acid in the embryo and in the whole grain, with the following results:

P_2O_5 in form of	Embryo		Seed	
	In 100 parts of dry matter	In 100 parts of total P_2O_5	In 100 parts of dry matter	In 100 parts of total F_2O_5
Lecithin	0.04	0.64	0.003	0.35
Phosphatides	0.22	3.54	0.018	1.86
Phytin	5.14	82.90	0.436	45.68
Inorganic salts	0.04	0.64	trace	——
Nucleic acids	0.76	12.28	0.502	52.61
Total	6.20	100.00	0.950	100.00

The above table shows that the embryo is very rich in phospooric acid of which the greater part is in the phytin-form.

(II) *Chemical properties of the embryo oil.*

The chemical constants of the embryo oil, extracted from the above sample with ether, are compared with the result obtained by Hirai⁽³⁾ and with that of polishing oil, reported by Takahashi⁽⁶⁾:

	Embryo oil		Polishing oil
	Present authors	Hirai	Takahashi
Acid value	26.5	29.74	14.36
Saponification value	183.0	212.19	183.54
Acetyl value	10.6	——	——
Iodine value	114.8	106.63	104.73
Hehner value	93.2	95.16	95.95
Reichert-Wollny value	0.4	2.05	0.73
Unsaponifiable matter	5.28%	4.00%	3.40%

The fatty acids obtained by saponifying the embryo oil with alcoholic potash in the usual way had the following constants:

m. p. 31~34°C; Acid value 196.3; Iodine value 109.9;
Mean molecular weight 283; Liquid fatty acids 75%.

(III) *Phytosterolin (phytosterine-glucoside).*

When the etherial extract of the rice-embryo was left standing for several hours, a brown precipitate separated out, which when recrystallized from amyl alcohol formed white needles melting at 283~290°C and gave the Liebermann's sterine reaction as well as the Molisch's reaction for sugar. From the analysis as well as from other properties, this substance was proved to be identical with the phytosterolin isolated from wheat-embryo by Nakamura and Ichiba(7).

Analysis of the phytosterolin from rice-embryo:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.752	10.005	3.464	72.73	10.33
II	3.711	9.847	3.415	72.37	10.29
Calculated for phytosterolin ($C_{33}H_{55}O_6$)				72.30	10.20

Like other sterines, this substance forms an acetate melting at 167~168°C.

Analysis of the acetate:

Substance (mg)	CO_2 (mg)	H_3O (mg)	C (%)	H (%)
4.378	11.059	3.503	68.90	8.95
Calculated for $C_{14}H_{64}O_{10}$			68.67	6.94

It forms also a benzoate melting at 198°C.

Analysis of the benzoate:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
3.131	8.726	2.169	76.01	7.75
Calculated for $C_{61}H_{72}O_{10}$			75.90	7.52

(IV) *Separation of saturated fatty acids.*

Rice-embryo (1.5 kg) was boiled with 3.5 litres of 90% alcohol for three hours, filtered while hot and the residue was extracted three times each with 2.5 litres of 90% alcohol. The combined extract was allowed to stand overnight, whereby a flocky precipitate separated out which chiefly consisted of waxes. It was filtered off and the filtrate was evaporated in vacuum and the residue was extracted with ether. The etherial extract was, after evaporating off the ether, saponified with alcoholic potash according to the usual method. From the mixed fatty acids thus obtained the solid saturated acids were separated by Tortelli Ruggeri's method. They had the following properties:

m. p. 60~61°C; Mean molecular weight 270; Acid value 208;
Iodine value 5.

The solid acids were now mixed with sand and dried on a waterbath for ten days and extracted with ether. The etherial solution was evaporated, the residue was again dissolved in petroleum ether, filtered, and the filtrate was evaporated, whereby, 100 g saturated acids were obtained.

For the isolation of each fatty acid, the above acid mixture was converted into methyl ester and subjected to fractional distillation. For this purpose the mixture was dissolved in 220 cc methyl alcohol, treated with dry HCl gas until the solution absorbed about 8 g of the acid and after heating for two hours on a water-bath the solution was shaken with ether. The etherial extract was, after washing with water to remove the methyl alcohol and hydrochloric acid, fractionally distilled under diminished pressure (8 mm). In this way the following fractions were obtained:

Fraction	Temperature (°C)	Yield (g)
I	160~176	1.5
II	176~186	80.5
III	186~213	7.2
IV (residue)	——	15.5

Each fraction was now saponified and free fatty acids thus regenerated had the following constants:

	Yield (g)	Acid value	Mean mol. wt.	m. p. (°C)
I	1.2	220.6	254.2	53~54
II	75.0	216.8	258.7	58~59
III	6.5	212.8	263.6	59~60
IV	12.3	——	——	——

The fraction I was recrystallized from 90% alcohol and the residue from the mother liquor, after evaporating the alcohol, was recrystallized twice from 80% alcohol. Again, the residuals from the combined mother liquors were recyrstallized twice from 70% alcohol. Then, evaporating off the combined mother liquors, the residual was dissolved in hot 70% alcohol, decolorized with animal charcoal and allowed to stand overnight. Thereupon, a crystalline precipitate separated out which melted at 54°C and had the mean molecular weight 234. Most probably it was myristic acid containing some impurities.

The fraction II, when recrystallized four times from 95% alcohol, melted at 62~63°C and had the acid value 218.8 and the mean molecular weight 256.3. The analysis gave the following results:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4.315	11.900	4.853	75.22	12.58
II	4.001	11.000	4.487	74.98	12.55
		Calculated for $C_{16}H_{32}O_2$		74.92	12.59

The analysis shows that it was palmitic acid.

The fraction III was coloured slightly yellowish, so it was dissolved in alcohol and after being decolorized with animal charcoal, recrystallized fifteen times from absolute alcohol. The crystals thus obtained melted at 59∼61°C and had the acid value 197.8 and the mean molecular weight 282. It was proved to be a mixture of stearic and palmitic acids, but the yield was too small for further purification.

The fraction IV was dissolved in 500 cc absolute alcohol and allowed to stand for 48 hours. The precipitate formed thereby was collected and washed with absolute alcohol: the yield, 3.4 g. After recrystallization from absolute alcohol eight times more, the crystals obtained melted at 76.5∼77.5°C with the acid value 141.1 and the mean molecular weight 398.2. The analysis gave the following results:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.903	11.209	4.533	78.33	12.99
II	3.587	10.301	4.250	78.33	13.25
		Calculated for $C_{26}H_{52}O_2$		78.61	13.13

The analysis shows the agreement with cerotic acid. The filtrates of cerotic acid were united, evaporated and the residue was recrystallized three times from absolute alcohol. The fatty acid obtained in this way had the melting point 76°C and the mean molecular weight 318. From this it was concluded that arachidic acid was present; but it could not be purified.

The mother liquor of fraction IV was diluted to 90% alcohol, the precipitate formed thereby was dissolved by heating and allowed to stand for three days, when the precipitate was collected, recrystallized from 90% alcohol and the filtrate therefrom was evaporated and the residue was recrystallized from 90% alcohol five times. The fatty acid thus obtained was long needles melting at 79.5∼80°C with the acid value 164 and the mean molecular weight 342.

Analysis of the fatty acid:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4.374	12.429	5.121	77.50	13.10
II	4.942	14.031	5.716	77.43	12.94
		Calculated for $C_{22}H_{44}O_2$		77.57	13.03

The analysis shows the substance to agree with behenic acid.

(V) *Bromination of unsaturated fatty acids.*

Unsaturated fatty acids (150 g) were brominated in usual way and the bromination products were digested with 2 L of boiling petroleum ether (35∼55°C), whereby a clear solution was obtained leaving no insoluble residue.

On standing overnight, 73 g of solid bromides separated out. The mother liquor, when concentrated to 1/3 of its volume and allowed to stand for three weeks, gave 3 g more of solid bromides.

When the solid bromide thus obtained was recrystallized three times from 95% alcohol, it melted at 113°C and had the acid value 93.7 and the mean molecular weight 599.9.

Analysis of the soild bromide:

	Snbstance (mg)	AgBr (mg)	Br (%)
I	6.645	8.295	53.12
II	6.030	7.553	53.30
	Calculated for $C_{13}H_{32}O_2Br_4$		53.33

The analysis shows it to agree with the tetrabromide of linolic acid.

When the filtrate of the solid bromide was evaporated, another bromide having 41.3% bromine was obtained. Probably it was the dibromide of oleic acid mixed with some tetrabromide.

(VI) *Oxidation products of the unsaturated fatty acids.*

When the unsaturated fatty acids were oxidized with alkaline premanganate according to the Hazura's method, 11.3 g insoluble hydroxyacids were obtained. They were treated with ether and separated into the ether-soluble and insoluble parts. The former, when recrystallized from absolute alcohol, melted at 131°C with the acid value 176.3 and the mean molecular weight 316.7. This was proved to be dihydroxystearic acid.

Analysis of the dihydoxystearic acid:

Substance (g)	CO_2 (g)	H_2O (g)	C (%)	H (%)
0.0982	0.2462	0.09852	68.35	11.14
	Calculated for $C_{18}H_{36}C_4$		68.29	11.47

The ether-insoluble part, when recrystallized from absolute alcohol, melted at 173°C, its acid value being 160.9 and the mean molecular weight, 394.1. It was sativic acid.

Analysis of the sativic acid:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.352	7.620	3.036	62.00	10.13
II	3.611	8.178	3.391	62.07	10.35
		Calculated for $C_{18}H_{36}O_6$		62.07	10.35

Linusic or isolinusic acid could not be detected.

(VII) *Unsaponifiable matter.*

When 20 kg rice.embryo was extracted with benzene, about 4 kg dark brown oil was obtained. It was dissolved in 12 L ether, a little insoluble waxy substance was filtered off and evaporated. The oil thus obtained was saponified with alcoholic potash in the usual way and 150 g of an uusaponifiable substance were obtained. It was a light brown semi-solid mass having a characteristic odour and giving a strong Liebermann-Buchard's reaction as well as the colour reaction of ergosterine. By treating with acetone and absolute alcohol it was separated into two part, i. e. (A) a white amorphous solid (120 g) and (B) a brown syrup (30 g).

(A) From the spectrographic studies it was calculated that the amorphous solid contained about 0.4~0.5% ergosterine. When irradiated with ultra-violet rays and tested on rats, the existence of vitamin D was clearly demonstrated.

(1) Melissyl alcohol.

When the amorphous solid (A), mentioned above, was recrystallized several times from absolute alcohol until it gave no more Liebermann–Buchard's reaction, about 0.05 g crystals was obtained which melted at 83°C.

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
2.798	6.462	3.524	82.48	14.09
	Calculated for $C_{30}H_{62}O$		82.19	14.15

The analysis shows it to agree with melissyl alcohol.

(2) Dihydrositosterol.

The mother liquors of the last few fraction of the said recrystallization were combined and evaporated. About 30 g of the residue thus obtained were again repeatedly recrystallized from absolute alcohol, and after drying, it weighed 3.2 g; m. p. 132°C, $[\alpha]_D^{22} = -6.20°$. The unsaturated sterines were decomposed by the Anderson's method, and about 0.13 g dihydrositosterine (m. p. 144°C, $[\alpha]_D^{22} = +24.28°$) was isolated and identifiedl.

The sterol obtained from the combined filtrate of the above recrystallization melted at 138~140°C, $[\alpha]_D^{23} = -26°$. This was extracted three times with a large quantity of petroleum ether (50°C) and then it was converted into acetate; m. p. 115~11°C, $[\alpha]_D^{22} = -23.98°$.

(3) Tetrabromide of stigmasterine acetate.

When the acetate, mentioned above, was recrystallized ten times from absolute alcohol, the top fraction (12 g) melted at 138°C and had the rotation, $[\alpha]_D^{19} = -43.7°$. This fraction was brominated by the Windaus–Hauth method adding an excess of glacial acetic acid. The precipitate was collected on a filter, dissolved again in chloroform and thrown down by methyl alcohol; m. p. 204~205°C.

Analysis of the bromide:

	Substance (mg)	AgBr (mg)	Br (%)
I	5.235	4.970	40.40
II	8.330	8.030	41.00
	Calculated for $C_{32}H_{52}O_2Br_5$		40.58

The above result agrees with the tetrabromide of stigmasterine acetate.

Owing to the scarcity of the material, the free stigmasterine could not be obtained.

(4) Anderson's γ–sitosterine⁽⁴⁾.

The bromides in the mother liquor of the above tetrabromide were combined, dissolved in ether and fractionally precipitated with methyl alcohol. The main fraction thus obtained was an amorphous powder, melting at 130°C. It was debrominated by boiling with zinc dust and acetic acid, and an acetyl derivative (m. p. 143°C, $[\alpha]_D^{20} = -44.70°$) was prepared. By saponifying the latter, a free sterol (m. p. 148°C, $[\alpha]_D^{21} = -42.3°$) was isolated.

Analysis of the free sterol:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.013	12.270	3.950	83.39	12.30
	Calculated for $C_{29}H_{50}O$		83.96	12.24

The analysis shows that it agrees with Anderson' γ–sitosterol.

(5) A new sitosterol.

The sterol acetates descrbied above were combined and saponified. About 80 g free sterol thus obtained, was dissolved in hexane and fractionally precipitated by gradual cooling as follows:

	Temperature (°C)	Yield (g)	m. p. (°C)	D
I	21	19.5	135	−29.34° (21°C)
II	1	16.5	134	−23.93° (20°C)
III	−12	20.0	132	−18.46° (18°C)
IV (residue)		22.0	148	——

The author could not separate from the above fraction I, II, III any substance that had constant melting point and rotation. The last fraction (IV) was recrystallized from absolute alcohol ten times, from acetone twice and further from methyl alcohol fifteen times and a new sitosterol (m. p. 156°C, $[\alpha]_D^{23} = -14.485°$) was isolated; yield, 2.1 g. It was more readily soluble in organic solvents than other sterines.

Analysis of the new sitosterol:

	Substace (mg)	Co_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.269	10.040	3.495	83.76	11.96
II	4.144	12.690	4.510	83.52	12.18
		Calculated for $C_{27}H_{46}O$		83.85	12.01

The acetate of the new sitosterol was prepared by boiling the free sterol with acetic anhydride for 40 minutes in the usual way; m. p. 111°C, $[\alpha]_D^{23} = -9.87°$.

Analysis of the acetate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.472	10.370	3.608	81.46	11.63
II	3.849	11.467	3.949	81.25	11.48
	Calculated for $C_{29}H_{48}O_2$			81.21	11.30

The propionate was obtained by boiling the free sterol with propionic anhydride for 1.5 hours in the usual way; m. p. 106°C, $[\alpha]^{23}_{D} = -6.78°$

Analysis of the propionate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.003	8.992	3.177	81.66	11.84
II	3.610	10.800	3.750	81.59	11.62
	Calculated for $C_{30}H_{50}O_2$			81.74	11.60

The benzoate was obtained by adding benzoylchloride to the sterol dissolved in pyridine at 2°C; m. p. 129°C, $[\alpha]^{20}_{D} = +14.48°$.

Analysis of the benzoate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	2.981	9.071	2.885	82.99	10.83
II	3.660	11.178	3.577	83.29	10.93
	Calculated for $C_{34}H_{54}O_2$			83.21	10.28

The acetate mentioned above was brominated by the Windaus-Hauth method, but when a small amount of bromine was added, the solution became brownish green, liberating HBr and required 50% more bromine than the calculated quantity. When bromine was added at −13°C, the green colour never appeared, only turning brown. The percentage of bromine was found to be 31.89 and 32.27%. A portion of the sterol was reduced with hydrogen using platinum-black as the catalyst, but the reduction proceeded very slowly, so that the Liebermann-Buchard reaction never disappeared. The molecular formula of the sterol calculated from its analytical results corresponds to $C_{27}H_{46}O$.

From the above observation it was believed to be an isomer of Burian's sitosterol. The so-called Anderson's α-sitosterol was still a mixture of various sterols, as is shown in the following table:

	Free sterol		Acetate	
	m. p. (°C)	Rotation	m. p. (°C)	Rotation
α-Sitosterol	138~140	−23.41°	115~116	−23.91°
Author's new sterol	156	−14.485°	111	− 9.87°

(B) The brown syrup.

The brown syrup was subjected to steam distillation and a little oil having a characteristic odour was obtained, but the amount was so small that it could not be examined further. From the residue, when further distilled at high vacuum a hydrocarbon mixed with a little higher alcohol was obtained.

(VIII) *Wax.*

When rice-embryo was boiled with strong alcohol or benzene, then filtered while hot and the filtrate was left to stand for several hours, the waxy substance separated out as a greyish white flocky precipitate, or when the crude embryo oil was treated with ether, the waxy substance remained as an insoluble residue. These were combined and extracted with hot benzene in Soxhlet apparatus and the extract was precipitated with ether. By repeating this operation five times needle-shaped crystals melting at 82°C were obtained; yield, 3.2 g.

The analysis gave the following results:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4.000	12.055	4.954	82.19	13.86
II	3.840	11.642	4.858	82.17	14.07
		Calculated for $C_{55}H_{112}O_2$		82.06	14.03

The analysis shows that it was melissyl cerotate.

For further identification, this substance was saponified by boilling with 5% potassium alcoholate for 4 hours. After cooling, the mixture was treated with alcoholic $CaCl_2$. The calcium soap thus formed was filtered off, and the filtrate was completely dried and extracted with benzene. The benzene solution was evaporated and the residue was recrystallized from absolute alcohol. In this way hair-like needles melting at 85°C were obtained.

Analysis of the sample:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.100	12.400	5.230	82.48	14.27
	Calculated for $C_{30}H_{62}O$		82.08	14.28

The result agrees with melissyl alcohol.

The Ca-soap obtained as above, was decomposed with dilute hydrochloric acid (1 : 1) and cerotic acid was isolated as insoluble crystalline powder. After recrystallization from absolute alcohol, it melted at 78°C and had the mean molecular weight 394. Mixed with a pure specimen of cerotic acid, no depression of melting point was observed.

(XI) *Cerebroside.*

When rice-embryo, previously extracted with benzol, was boiled with 85 ~90% methyl alcohol and the methyl alcoholic solution was evaporated in vacuum to a syrupy consistency and left standing for several days, a solid substance separated out. It was collected by suction and dissolved in ether. The etherial solution thus obtained was, after washing with water, treated with acetone, whereby a precipitate was formed which gave ninhydrine and

Molisch's reactions. This precipirate was dissolved in ether and precipitated by adding absolute alcohol. By repeating this operation four times, a crystalline substance was obtained which gave no more the above reactions. When dried in vacuum desiccator it became brownish black and was no more soluble in ether. The mother liquor of the above crystals was shaken with water and the separated ether layer was dehydrated, evaporated to a small volume and treated with acetone, whereby a precipitate was formed, which when recrystallized from absolute alcohol gave ninhydrine and Molish's reactions. It resembled the cerebroside, obtained by Trier from rice grain. Owing to the scarcity of the material, it was not further investigated. Details concerning this part will be published later.

In conclusion, the authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his advices and encouragement throughout this work and also to Mr. T. Iki and T. Tanaka for micro-analysis.

Studies on the Fermentation products by Mould Fungi. Part X.

Glaucic Acid formed by Aspergillus glaucus.
(Aspergillus glaucus. IV.)

By

Yusuke SUMIKI.

(*Agricultural Chemical Laboratory, Tokyo Imperial University*).

(Received May 23, 1933).

On the previous report of this series (part IV), the author described that a new crystal substance, m. pt. 238~9°, was obtained by the cultivation of *Aspergillus glaucus* under a certain condition. Since, the author cultivated *Aspergillus glaucus* several times under the same condition but could not obtain this crystal substance. Unexpectedly, the author obtained this substance by treating the mycelium of *Aspergillus glaucus* with ether on account of the isolation of chitin from the mycelium. This new crystal substance was named glaucic acid after *Aspergillus glaucus*.

Glaucic acid melts at 238~9° and contains no nitrogen, methoxyl, ketomethylen and methylenoxide groups. The results of analyses of glaucic acid and its derivative show that the molecular formula of glaucic acid is $C_{17}H_{22}O_5$.

The existence of one carboxyl group in the glaucic acid is identified by the following experiments. Glaucic acid is soluble in Na–carbonate and precipitated by the addition of mineral acid. The results of titration with standard alkali, using phenolphthalein as the indicator, shows glaucic acid is a monobasic acid. Moreover glaucic acid is not methylated with dimethylsulphate but methylated with diazomethane and this monomethylderivative is insoluble in Na–carbonate and alkali.

By treating glaucic acid with benzoylchloride or acetic acid anhydride, the benzoyl- or acetyl-derivatives are not obtained. Moreover, the results of quantitative determination of hydroxyl group in glaucic acid by the method of Tschugaeff and Zerewitinoff shows the existence of one hydroxyl group and this fact indorses the existence of one carboxyl group.

Isolation and properties of glaucic acid.

The mycelium of Aspergillus glaucus grown on the medium which contains glucose, peptone and mineral matter is washed with water completely, dried at 100°, powdered and extracted with ether for a week. On evaporating off the solvent from the extract, glaucic acid crystallizes in prism. Yield : 1.0 g. of glaucic acid from 411 g. of mycelium. This crude substance is dissolved in dilute alkali, precipitated with hydrochloric acid, washed with hot water and then recrystallized from boiling alcohol. Glaucic acid, prisms, melts at 238∼9° and contains no nitrogen. The reactions of methoxyl group by micro-Zeisel, methylenoxide group by Tollen's phloroglucinol and ketomethylen group by Na-nitroprusside are all negative. It is soluble in Na–carbonate, alkali and precipitated by the addition of mineral acid. It is also easily soluble in boiling ethyl-, methyl-alcohols, acetone, difficultly in benzene, chloroform, acetic ester and nearly insoluble in boiling water.

Subst. (mg.)	CO_2 (mg.)	H_2O (mg.)	C %	H%
3.218	8.019	2.182	66.92	7.47
3.142	7.679	2.182	66.65	7.77
3.358	8.193	2.228	66.54	7.42

(g.)	Campher (g.)	ΔT (°)	M.W.
0.0110	0.1130	13	300
0.0133	0.1335	13	306
0.1129	$n/10$ NaOH 3.515 ccm.		304
0.0974	CH_4 5.6 ccm. (762.5 mm, 20°)		

	M.W.	C %	H%	O%	OH%
$C_{17}H_{22}O_5$ cal.	306	66.67	7.19	26.14	5.56
($C_{16}H_{21}O_3$ COOH) fou. (average)	303	66.70	7.42	25.74	4.39

Glaucic acid monomethyl ester : — 0.3 g. of glaucic acid is suspended in ether and added with ethereal solution of diazomethane prepared from 3 ccm. of nitrosomethylurethane. Yield : 0.31 g. The methylester is easily soluble in acetic ester, benzene, chloroform, difficultily in ethylalcohol, ligroine and insoluble in water, Na-carbonate and alkali. It is recrystallized from alcohol or ligroin. Needles. M. pt. 188~90°.

Subst. (mg.) 3.200	CO_2 (mg.) 7.873		H_2O (mg.) 2.234
〃 4.040	AgJ 2.673		
	C %	H%	OCH_3%
$C_{17}H_{21}O_4$ (OCH_3) cal.	67.50	7.50	9.69
foun.	67.10	7.80	8.74

Methylation with dimethylsulphate :— 0.2 g. of glaucic acid is dissolved in 40 ccm. of methylalcohol and shaked vigorously for 20 minutes adding 1 ccm. of dimethylsulphate and 2 ccm. of 25% kaliumhydroxide. After repeating this procedure 5 times, the reaction mixture is evaporated, acidified and filtered. Yield : 0.15 g. It is recrystallized from benzene. Prisms. M. pt. 234~5°. The admixture with glaucic acid melts at 236~7°.

Subst. (mg.)	CO_2 (mg.)	H_2O (mg.)
3.394	8.218	2.270
	C %	H %
$C_{17}H_{22}O_5$ cal.	66.67	7.19
foun.	66.40	7.48

Acetylation and benzoylation :— 0.2 g. of glaucic acid is added with 5 g. of acetic acid anhydride and 0.5 g. of dehydrated Na-acetate and boiled for 4 hours under the reflux condenser in the oil bath. The reaction mixture is poured into water and the precipitate is filtered. Yield : 0.2 g. It is recrystallized from dilute methylalcohol. Prisms. M. pt. 234~5°. The admixture with glaucic acid also melts at 235~6°.

0.2 g. of glaucic acid is dissolved in pyridine and added with 0.4 g. of benzoylchloride. After several days, the reaction mixture is poured into water and the precipitate occured is filtered. Yeild : 0.2 g. It is recrystallized from alcohol. Prisms. M. pt. 234~5°. The m. pt. of admixture with glaucic acid shows no depresssion.

Biochemistry of Filamentous Fungi. II.

A Metabolic Product of Aspergillus melleus Yukawa. Part I.

By

Hidejiro NISHIKAWA.

(Received May 31, 1933)

As is well known, filamentous fungi emit more or less peculiar mouldy smell, differing in nuance and intensity according to species or varieties ; some produce even agreeable odour, as, for instance, Aspergillus oryzae. The chemical nature of their smell has, however, been little investigated, nothing definite being known as it. It is presumably due to mixture of minute quantities of diverse substances and consequently it will in general be tedious task to isolate each constituent in sufficient quantity for chemical investigation. But researches in this line will provide some scientific interest and be not without significance for the physiology of mould fungi.

The author has isolated from the culture medium of Aspergillus melleus a crystalline substance which is supposed to be a constituent of the smell of the mould. It was provisionally named mellein. Analysis of mellein and its nitro-derivative show that its probable formula will be $C_{10}H_{10}O_3$. Purple coloration with $FeCl_3$, positive Millon's reaction, and precipitation with bromine water from aqueous solution indicate its phenolic character.

In 1931 and 1932 a substance, $C_{14}H_{14}O_4$, m.p. 58.5°, was isolated and described by Yamano(1) from cultural solution of Aspergillus ochraceus. This substance and mellein which melts at 58° have almost the same melting point ann their properties and reactions are quite similar. As Asp. melleus and ochraceus have close morphological resemblance and according to Thom and Church(2) Asp. melleus is to belong to Asp. ochraceus group, possible identity or at least strong similarity in structure of both substances could be anticipated.

Experimental.

Preparation of mellein.

The strain of Aspergillus melleus used in the present investigation was purchased from the Centraalbureau voor Schimmelcultures, Baarn, Holland. The culture medium was of the following composition : —

Sucrose (commercial)	50 g.	$MgSO_4 \cdot 7H_2O$	0.5 g.
$NaNO_3$	2 g.	$FeSO_4 \cdot 7H_2O$	0.01 g.
KH_2PO_4	1 g.	H_2O	1000 c.c.
KCl	0.5 g.		

The above medium contained in Erlenmeyer flasks was sterilized and conidia of Asp. melleus were abundantly inoculated in it. After 2 to 3 weeks of incubation at 30° the medium was filtered free from mycelial felt, saturated with common salt and extracted with ether. The ether extractives were then submitted to steam distillation and the distillate was again extracted with ether. When ether was driven off, the residue solidified to crystalline magma. Yield, 0.3 g. from 1 litre of the medium, corresponding to 0.6 per cent. of the sugar used. This is already fairly pure. The specimen purified by disolving in alkali and reprecipitating with acid was submitted to analysis.

Anal. Subst.$=0.1850$; $CO_2=0.4578$; $H_2O=0.0968$ g.

Found : $C=67.49$; $H=5.81\%$

Calc. for $C_{10}H_{10}O_3$: $C=67.42$: $H=5.62\%$.

Mol. wt. 0.2054 g. subst. in 21.98 g. benzene. (Cryoscopy).

$d=0.281°$, $M=162.4$. $C_{10}H_{10}O_3=178$

Rotatory power. 0.1988 g. subst. in 25 c.c. chloroform.

$\alpha_D^{12}=-0.86°$. $[\alpha]_D^{12}=-108.15°$.

Mellein crystallizes in colourless long prisms, sparingly soluble in water, soluble in ethyl and methyl alcohol, chloroform, ether, and light petroleum. Purple coloration with $FeCl_3$ is marked in 1/10,000, faintly visible even in 1/100,000 dilution. From solution in alkali mellein is precipitated by passing CO_2.

Nitration of mellein :— Mellein was dissolved in conc. nitric acid and stood over nicht. On addition of water white flocky precipitate separated out. Pale yellow beautiful needles with greenish tint were obtained when recrystallized from methyl alcohol.

Anal. Subst.$=0.1720$; $CO_2=0.3404$; $H_2O=0.0652$ g.

Subst.$=0.2276$; $N_2=12.8$ c.c. (19.5°, 766 mm).

Found : $C=53.97$; $H=4.21$; $N=6.47\%$.

Calc. for $C_{10}H_9O_5N$: $C=53.81$; $H=4.04$; $N=6.28\%$.

Rotatory power. 0.1996 g. subst. in 25 c.c. Chloroform.

$\alpha_D^{19}=-1.37°$. $[\alpha]_D^{19}=-171.59°$.

Analysis agree with a mononitro-derivative af mellein. It melts at 183 ∼184° after turned yellow in the neighbourhood of 140°. It is insoluble in ether and light petroleum, sparingly soluble in water, soluble in benzene, methyl and ethyl alcohol, readily soluble in acetone and chloroform. It gives an intensely yellow solution when dissolved in alkali and separates out as white precipitate by acid. Its aqueous solution turns light red, ethyl alcoholic solution blood red on addition of $FeCl_3$.

References.

(1) Yamano : Jôzôgaku Zasshi, Vol. 9, p. 514, (1931).
〃 Jôzôgaku Zasshi, Vol. 10, p. 528, (1932).
(2) Thom & Church : The Aspergilli, 1926, p. 73 & 188.

Studies on the Calcium and Magnesium Absorption of Rice Plants at Different Stages of their Growth in Water Culture.

By

Yoshiaki ISHIZUKA.

(Agricultural Chemical Laboratory Faculty of Agriculture, Hokkaido Imperial University)

(Received June 26, 1933)

Résume.

Experiment on the calcium and magnesium absorption of rice plants at different stages of their growth was conducted with water culture as a means of elucidating their nutritional physiology. The conclusions obtained are as follows : —

(1) The presence of calcium in nutrient solution is absolutely necessary for rice plants till the 13th week after germination in order to assure their attaining maturity. After that time, the lack of calcium has no harmful effects on the growth, as seen by a comparison of plants under calcium-free nutrients with the control, which is supplied with complete nutrients through the entire period of growth. But the earlier the time of the calcium deficiency in nutrient solution within 13 weeks after germination, the more hindered was the growth of rice plants.

The presence of magnesium in nutrient solution is necessary and sufficient for the full growth of rice plants till the 7th week after germination. So their growth is not hindered by the lack of magnesium after that time.

W. F. Gericke concludes in his essay "the absence of one or more of elements calcium and magnesium after initial exposure of rice plants to the complete nutrient solution, did not have any pronounced harmful effect on the development." and in the case of calcium, his data show that the period of initial exposure is about 6 weeks ; but the writers experiment shows that, in the case of calcium, its presence for 13 weeks in nutrient solution is inevitably necessary for the growth of rice plants. In the case of magnesium, the writers result almost agrees with that of Gericke.

(2) The growth of rice plants is not affected by the lack of calcium in nutrient solution till the 5th week after germination, if there is a supply of calcium after that time. But the earlier the time of supply, the better the recovery of the growth which is badly influenced by calcium deficiency. When a supply of calcium was lacking for more than 6 weeks after germi-

nation, the growth could no more recover in spite of giving a supply of calcium. In most serious cases, plants died.

The lack of magnesium till the 6th week after germination dose not affect the development of the plants except for a little ill effect on seed formation. However, the earlier the time of magnesium supply, the better the growth of plants.

(3) As the presence of calcium is necessary for the development of rice plants through almost all stages of growth, it is hard to show when calcium is utilized most significantly. But it is supposed to be from the 5th to the 7th week after germination.

In the case of magnesium, the maximum point of utilization is supposed to lie between the 4th and 8th week after germination.

(4) The ratio of CaO to MgO absorbed by rice plants is about 3 : 1 in weight at an early period of their growth ; it gradually approaches to 1 : 1. However, the ratio of CaO to MgO absorbed by plants in the entire period of their growth is 1.2 : 1.0 in weight.

Sterilizing Action of Acids. IV Report.

Sterilizing Action of Unsaturated Monobasic Fatty Acids on Putrifactive bacteria, Bac. typhosus, and Vib. cholerae.

By

Sogo TETSUMOTO.

(Received June 14, 1933).

Contents

(1) Reagents.
(2) Experimental methods.
(3) Sterilising action at the same molecular concentration.
(4) Sterilizing action of anions.
(5) Comparison of sterilizing action between saturated and unsaturated monobasic fatty acids of the same molecular concentration.
(6) Sterilizing action at the same pH of monobasic fatty acids.
(7) Summary.

(1) Reagents.

Details of the sterilizing action of saturated monobasic fatty acids $C_nH_{2n+1}CO_2H$ are as reported before.

To know the sterilizing action of unsaturated monybasic fatty acids on bacteria by comparing it with the action of the saturated fatty acid series, I used the following acids.

Table I.—Reagents. $C_nH_{2n-2}CO_2H$.

Acids	numbeo of C atom	Rational formulae	Molecular weight	PH at N/100	PH at N/1000
Acrylic acid	C_3	$CH_2:CH\cdot CO_2H$	72.047	3.13	3 63
nor. Crotonic acid	C_4	$CH_2:CH\cdot CH_2\cdot CO_2H$	86.068	3.33	3.83
Undecylenic acid	C_{11}	$CH_2:CH\cdot(CH_2)_8\cdot CH_2H$	184.071	saturated aq. solution	5.4
Oleic acid	C_{18}	$CH_3\cdot(CH_2)_7CH:CH(CH)_7CO_2H$	282.362	〃	6.0

acrylic acid··················made by Fraenkel & Landau Co.
other 3 acids ················S. Kahlbaum Co.

Undecylenic acid and oleic acid are scarcely soluble in water, so I made saturated aqueous solution of these acids at 20°C.

(2) Experimental methods.

Experimental methods and used microorganisms and e.t.c. are the same as I reported before(1)(2).

(3) Sterilizing action at the same molecular concentration.

To know the sterilizing action at the same molecular concentration of unsaturated monobasic fatty acids on Staph. c. pyogen. aureus, Proteus vulgar., Hauser, Bac. typhosus and Vib. cholerae, I studied the sterilizing action of *N*/100 and *N*/1000 solutions of acrylic acid and normal crotonic acid, and saturated aqueous solution of undecylenic acid and oleic acid at 20°C. Results are as shown in the following table.

Table 2.—Sterilizing action at *N*/100.

Number of C atom	acids	pH	Surviving period															
			Staph. c. pyog.				Prot. vulgar.				Bac. typhosus				Vib. choler			
			h 3	6	9	12	m 60	90	h 2	3	m 60	90	h 2	3	m 5	10	15	20
C_3	Acrylic	3.1	+	+	−	−	+	+	−	−	−	−	±	−	+	±	−	−
C_4	nor. Crotonic	3.3	+	+	±	−	+	+	±	−	+	+	+	−	+	+	+	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

m·········minut, h·········hour, +·········alive, −·········perished,
±······sometimes alive and sometimes perished.

Table 3.—Sterilizing action at *N*/1000 and saturated aqueous solution.

Number of C atom	Acids	pH	Surviving period																											
			Staph. c. pyogen							Prot. vulgar.							Bac. typhosus							Vib. choler.						
			m 60	90	h 12	24	36	d 5	6	m 30	45	h 9	12	d 2	3	4	m 45	60	h 12	24	36	d 3	4	m 1	2.5	45	60	90	h 2	3
C_3	Acrylic	3.6	+	+	+	−	−	−	−	+	+	+	−	−	−	−	+	+	+	−	−	−	−	+	+	±	−	−	−	−

C_4	Crotnic	3.8	+	+	+	+	−	−	−	+	+	+	±	−	−	−	+	+	+	±	−	−	−	+	+	+	−	−	−	−
C_{11}	Undecylenic	5.4	+	−	−	−	−	−	−	±	−	−	−	−	−	−	±	−	−	−	−	−	−	±	−	−	−	−	−	−
C_{18}	Oleic	6.0	+	+	+	+	+	±	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	±	−	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

d……day.

From results noted in Table 2 and 3, we find the following facts. At the same molecular concentration of unsaturated monobasic fatty acids, acrylic acid (C_3) is stronger than crotonic acid (C_4) as concerns to the sterilizing action. In the saturated aqueous solution at 20°C, undecylenic acid (C_{11}) has been dissolved in an extraordinarily small quantity but its sterilizing action is extremely strong. The sterilizing action of oleic (C_{18}) is very weak.

(4) Sterilizing action of anions of unsaturated monobasic fatty acids.

To know the sterilizing action of anions of each acid, I made neutral salts of Na, K, Ca and NH_4 having the same anions of each acid. Concentration of salts of acrylic acid and crotonic acid are *N*/100. And salts of undecylenic acid and oleic acid are in saturated aqueous solution at 20°C. respectively. The results are as shown in Table 4. The results of K salts are nearly the same as Na salts, so I denote the results of Na salts only.

Table 4.—Sterilizing action of anions of unsaturated monobasic fatty acids.

(I)—Na salts.

Na-	Surviving Period																			
	Staph. c. pyogen.						Prot. vulgar. H.					Bac. typhosus					Vfb. choler			
	h 6	9	d 5	d 6	7	8	h 2	3	d 4	5	6	h 3	6	d 5	6	7	m 2.5	5	h 3	6
acrylate	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	±	−	−	−	−	−	±	−	−	−	−	+	−	−	−	−	+	−	−	−
oleiate	+	+	+	−	−	−	+	+	+	−	−	+	+	±	−	−	+	+	+	−
Control	+	+	+	+	+	+	+	+	+	±	−	+	+	+	±	−	+	+	+	+

(II)—Ca salt.

Ca-	Surviving period																			
	Staph. c. pyogen						Prot. vulgar. H.					Bac. typhosus					Vib. choler.			
	h 4	6	d 5	6	7	8	m 90	h 2	d 3	4	5	h 3	6	d 5	6	7	m 2.5	5	h 3	3
acrylate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	+	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−	±	−	−	−
oleiate	+	+	+	+	−	−	−	+	+	+	−	+	+	±	−	−	+	+	±	−
Control	+	+	+	+	+	+	−	+	−	+	±	+	+	+	±	−	+	+	+	+

(III)—NH_4 salts.

NH_4-	Surviving Period																			
	Staph. c. pyogen						Prot. vulgar. H.					Bac. typhosus					Vib. choler.			
	h 9	12	d 6	7	9	10	h 3	6	d 5	6	7	h 6	9	d 6	7	8	m 2.5	5	h 6	9
acrylate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	±	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−	+	±	−	−
oleiate	+	+	+	−	−	−	+	+	+	−	−	+	+	±	−	−	+	+	±	−
Control	+	+	+	+	−	−	+	+	±	−	−	+	+	±	−	−	+	+	+	+

From the results shown in Table 4 (I), (II), (III), we find the following facts. Salts of acrylic acid (C_3) and crotonic acid (C_4) have no sterilizing action. Salts of undecylenic acid (C_{11}) have very strong sterilizing action. Salts of oleic acid (C_{18}) have very weak sterilizing action compared with the control.

These salts are all in neutral aqueous solution and have the same anions as acids. By these facts we know following facts.

Anions of acrylic acid (C_3) and crotonic acid (C_4) have no sterilizing action. Anion af undecylenic acid (C_{11}) has an extremely strong sterilizing action. Anion of oleic acid (C_{18}) has very weak sterilizing action.

Among salts of Na, K, Ca and NH_4, Ca salts have a somewhat short surviving period and NH_4 salts have a somewhat long surviving period for bacteria.

(5) Comparison of sterilizing action between saturated and unsaturated of monobasic fatty acids at the same molecular concentration.

I compared the sterilizing action of each acids having the same number of C atoms, at the same molecular concentration and in saturated aqueous solution at 20°C.

The results are as shown in the following table. (Table 5, (I), (II).)

Table 5.—Comparison of the sterilizing action between saturated and unsaturated monobasic fatty acids.

(I)—Results at $N/100$.

Number of C atom	Microorganisms		Staph. c. pyog.				Prot. vulgar. H.				Bac. typhosus					Viv. cholerae				
	Acids	time / PH	h 6	9	12	24	m 90	h 2	3	6	m 90	h 2	3	6	9	m 5	10	15	20	30
C_3	Propionic	3.4	+	+	−	−	+	+	−	−	+	+	±	−	−	+	+	+	−	−
	Acrylic	3.1	+	−	−	−	+	−	−	−	+	±	−	−	−	+	±	−	−	−

C_4	Butyric	3.4	+	+	−	−	+	+	−	−	+	+	±	−	−	+	+	+	−	−
	Isobutyric	3.4	+	+	+	−	+	+	+	−	+	+	+	±	−	+	+	+	+	−
	Crotonic	3.3	+	±	−	−	+	±	−	−	+	+	−	−	−	+	+	+	−	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(II)—Results at *N*/1000 and saturated aqueous solution at 20°C.

Number of C atom	Microorganisms		Staph. c' pyogen. aur									Prot. vulgar. Hauser								
	Acids	PH \ time	m 60	90	h 12	24	36	d 2	5	6	9	m 30	45	h 9	12	24	36	d 3	5	7
C_3	Propionic	3.9	+	+	+	+	±	−	−	−	−	+	+	+	+	−	−	−	−	−
	Acrylic	3.6	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−
C_4	Butyric	3.9	+	+	+	+	±	−	−	−	−	+	+	+	+	−	−	−	−	−
	Isobutyric	3.9	+	+	+	+	+	+	−	−	−	+	+	+	+	±	−	−	−	−
	Crotonic	3.8	+	+	+	+	−	−	−	−	−	+	+	+	±	−	−	−	−	−
C_{11}	Undecylic	6.0	+	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−
	Undecylenic	5.4	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
C_{18}	Stearic	6.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Oleic	6.0	+	+	+	+	+	+	±	−	−	+	+	+	+	+	+	+	−	−
	Control		+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	±	−

Number of C atom	Microorganisms		Bac. typhosus									Vib. cholerae								
	Acids	PH \ time	m 45	60	h 12	24	36	d 3	4	5	9	m 1	2.5	5	45	60	90	h 2	3	6
C_3	Propionic	3.9	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	Acrylic	3.6	+	+	±	−	−	−	−	−	−	+	+	+	±	−	−	−	−	−
C_4	Butyric	3.9	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	Isobutyric	3.9	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
	Crotonic	3.8	+	+	+	±	−	−	−	−	−	+	+	+	+	+	−	−	−	−
C_{11}	Undecylic	6.0	+	+	+	+	−	−	−	−	−	+	±	−	−	−	−	−	−	−
	Undecylenic	5.4	+	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−
C_{18}	Stearic	6.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	Oleic	6.0	+	+	+	+	+	+	+	−	−	+	+	+	+	+	±	−	−	−
	Control		+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+

From the results noted in Table 5 (I), (II), we ascertained the following facts. If we compare the sterilizing power at the same molecular concentration, we find that unsaturated monobacic fatty acids have stronger sterilizing action than saturated monobasic fatty acids.

We find also in saturated aqueous solution at 20°C, unsaturated monobasic fatty acids have stronger sterilizing action than saturated monobasic fatty acids. This phenomena is especially distinctly by C_{11} acids.

(6) Sterilizing action at the same pH of monobasic fatty acids.

To find the sterilizing action at the same pH, and to compare the sterilizing action at the same molecular concentration of monobasic fatty acids, I made solutions of pH 3.0 and pH 4.0 with each acid, and compared them at the same number of C atom. Acids of C_{11} and C_{18} were compared at pH 6.0 and pH 6.2 respectively. Results are as shown in Table 6. (I), (II).

To make the solution of pH 6.0 with saturated undecylenic acid solution of pH 5.4, we must dilute the acid with distilled water 20 times by volume. Also to make the solution of pH 6.2 with oleic acid of pH 6.0, we must dilute the acid with distilled water 5 times by volume.

Table 6.—Sterilizing action at the same pH of monobasic fatty acids.

(I)—pH 3.0

Number of C atom	Microorgnism		Staph. c. pyog.				Prot. vulgar. H.				Bac. typhosus.				Vib. cholerae			
	Acids	time / PH	h 2	3	6	9	m 45	60	90	h 2	m 60	90	h 2	3	m 1	2.5	5	10
C_3	Propionic	N/20	+	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−
	Acrylic	N/80	+	+	+	−	+	+	±	−	+	+	±	−	+	+	+	−
C_4	Butyric	N/20	+	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−
	Isobutyric	〃	+	+	−	−	+	+	−	−	+	+	+	−	+	+	−	−
	Crotonic	N/40	+	+	±	−	+	+	±	−	+	+	+	−	+	+	±	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(II)—pH 4.0, pH 6.0, pH 6.2

Number of C atom	Microorganism		Staph. c. pyogen.								Prot. vulgar. H.							
	Acids	time / conc.	h 9	12	36	d 2	3	4	5	8	h 2	3	12	24	36	d 2	5	6
C_3	Propionic	N/2000	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−
	Acrylic	N/8000	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	−
C_4	Butyric	N/2000	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−
	Isobutyric	〃	+	+	+	+	−	−	−	−	+	+	+	±	−	−	−	−
	Crotonic	N/4000	+	+	+	+	±	−	−	−	+	+	+	+	±	−	−	−
C_{11}	Undecylic	satur.	+	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−
	Undecylenic	〃	±	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−

C_{18}	Stearic	satur.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	Oleic	〃	+	+	+	+	+	+	±	−	+	+	+	+	+	+	−	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−

Number of C atom	Microorganism		Bac. typhosus.								Vib. cholerae							
	Acids	time / conc.	h 6	12	24	36	d 2	3	4	7	m 5	10	30	60	90	h 2	3	3
C_3	Propionic	N/2000	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−
	Acrylic	N/8000	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
C_4	Butylic	N/2000	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−
	Isobutyric	〃	+	+	+	+	−	−	−	−	+	+	+	+	+	−	−	−
	Crotonic	N/4000	+	+	+	+	±	−	−	−	+	+	+	+	+	−	−	−
C_{11}	Undecylic	satur.	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−
	Undecylenic	〃	±	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−
C_{18}	Stearic	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±
	Oleic	〃	+	+	+	+	+	+	−	−	+	+	+	+	±	−	−	−
	Control		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+

From the results noted in Table 6. (I), (II), we find the following facts. If we compare the sterilizing power of C_3 and C_4 at the same pH, we find that unsaturated acids have weaker sterilizing power than saturated acids. And then anions of these acids have no sterilizing power. (Table 4. (I), (II).) Accordingly, the molecular concentration of these acids has a great effect on sterilization. Concerning the sterilization of acids of C_{11} and C_{18}, unsaturated monobasic fatty acids have stronger sterilizing power than saturated acids, even if unsaturated acids, are adjusted to the same pH. And these anions have sterilizing power respectively. But if we compare the results noted in Table 3 and Table 6, we find that undissociated molecules of these acids have considerable sterilizing power.

Summary.

I studied the sterilizing action of unsaturated monobasic fatty acids on putrifactive bacteria, Bac. typhosus and Vib. cholerae. Results are as follows.

(1) At the same molecular concentration, acrylic acid (C_3) and crotonic acid (C_4) have nearly the same sterilizing power. But in detail, acrylic acid has the slightly stronger sterilizing power than crotonic acid. Undecylenic acid (C_{11}) is very slightly soluble in water, but its sterilizing action is very strong.

(2) Anions of C_3 and C_4 acids have no sterilizing action.

(3) Anions of C_{11} acid has strong sterilizing action.

(4) At the same molecular concentration, unsaturated monobasic fatty acids have stronger sterilizing power than saturated manobasic fatty acids. This fact is distinct by C_{11} acid.

(5) At the same pH of acids of C_3 and C_4, saturated monobasic fatty acids have stronger sterilizing power than unsaturated acids.

(6) Molecular concentration and undissociated molecules of these acids have a considerable sterilizing power.

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Biochemical Studies on "Miso", Fermented Soy-bean Paste. Part I.

Nutritive Value of "Miso"–Proteins as a Supplement of Rice.

By

Iwao IWAMURA.

(*Agricultural Chemical Laboratory, Tōkyō Imperial University, Komaba, Tōkyō.*)

(Received July 10, 1933.)

Introduction.

For more than a thousand years "miso" has been used as one of the most important food articles in Japan. Boiled rice and "miso"-soup form an indispensable daily ration for the Japanese people. "Miso" is also largely used with vegetables, fish and meats. Its annual production in recent years is estimated to be worth more than twenty millions of yen, nevertheless the role it plays in nutrition is yet little understood, so that more thorough study of this subject is desirable.

O. Kellner(1) was the first who analysed various kinds of "miso" and pointed out that it was rich both in true albuminoids and soluble carbohydrates and that it was easily digestible and nutritious. Later T. Takahashi(2) investigated the decomposition products of proteins in "Hatchōmiso" (brown miso) and isolated alanine, leucine, proline, glutaminic acid and lysine; besides detecting the presence of aspartic acid, histidine, arginine, tryptophane and cystine.

T. Hara and R. Takata(3) have recently carried out feeding experiments with white rats and observed that the rats fed on a diet in which "miso" had been added as the sole source of proteins, failed to grow or to sustain life for a long period. They have attributed this to the lack of certain indispensable amino acids, such as cystine and tryptophane in "miso", which might have been partly destroyed during the fermentation process. We see, however, from the chemical analysis that nearly half of the nitrogen contained in "miso" still exists in the form of proteins, even after a long period of fermentation. Recent investigation has further proved that the amino-acids and bases formed by the decomposition of proteins can be again utilized for the formation of body proteins, so it is very probable that these nitrogen compounds contained in "miso" still play an important role in nutrition. Thus we can expect a still better result than that mentioned above, when "miso" is used, not as the sole source of proteins in the diet, but as the supplement of rice proteins.

From such a point of view, the present author has carried out feeding experiments with various kinds of "miso" to determine their relative nutritive value, especially when used as the supplement of rice.

The details are given as follows : ——

Experimental.

I. Experiment with "Sendaimiso".

The sample used in this experiment was supplied from a "miso" factory in Tōkyō. This kind of "miso" may be taken as standard because it is most widely used and seems to be especially favoured in Tōkyō and in the north-eastern provinces.

It was prepared from 100 parts by volume, of soy-beans; 50 parts rice "koji" (steamed polished rice moulded by a special fungus, Aspergillus oryzae) and 50 parts common salt. The soy-beans were first soaked in water for several hours and steamed (at one pound pressure) for about ten hours until they turned brown. After cooling, they were finely ground, mixed with rice "koji" and salt put in a fermenting tank, covered with a wooden lid, pressed and kept in a cold place. Owing to the presence of a large quantity of common salt and the prevention of the free access of air, the fermentation proceeds very slowly and is only completed after 12 to 18 months.

Recently Y. Sakurai(4) investigated in our laboratory the changes in chemical composition during the ripening of "Sendaimiso" and obtained the following results :

Table I. Changes of the Chemical Composition of "Sendaimiso" during Fermentation. (%)

Composition	Before fermentation	Time of fermentation (Months)				
		1	3	6	9	12
Water	48.52	51.25	50.64	50.20	50.90	52.92
Total–N	1.98	2.10	2.08	2.19	2.08	2.14
Protein–N	1.44	1.28	1.07	1.08	1.03	0.98
N–insol. in water	1.26	1.17	0.99	1.02	0.97	0.94
N–sol. in water	0.72	0.93	1.09	1.17	1.11	1.20
Amino–N	0.29	0.42	0.52	0.51	0.50	0,49
Basic–N	0.24	0.27	0.29	0.31	0.32	0.30
NH_3–N	0.041	0.075	0.100	0.110	0.095	0.110
Reducing sugar	8.95	9.94	9.36	9·60	9.63	9.49
Starch & dextrin	4.92	3.00	——	——	2·41	2.40
Free acid (as lactic)	1.48	1.39	1.93	1.81	1.93	2.32

From the above table it can be seen that the proteins are gradually dissolved and decomposed until after 12 months they are reduced to 2/3 of the original sample. On the contrary there is a considerable increase of water-soluble nitrogen, including amino, basic and ammoniacal nitrogen. As the proteins in the material are denaturated by heat and fermentation and a part of the amino acids and bases is further decomposed into lower compounds which finally give rise to ammonia, it is natural to expect that the nutritive value is more or less decreased, though it may in some degree be compensated with the increase of digestibility. The chief object of the present investigation is to determine the effect of proteins in various kinds of "miso" upon the growth of rats when used as the supplement of rice, and at the same time to compare them with the proteins of "kōridōfu" (frozen soy-bean-curd) and fish meat powder.

Experiment (A).

Table II. Diets used in the Experiment. (g)

Composition of diets	(I) Control group	(II) Miso group	(III) Kōridōfu grop	(IV) Fish powder group
Polished rice	94.0	79.0	85.8	88.2
Sendaimiso	——	20.0*	——	——
Kōridōfu	——	——	9.2	——
Fish meat powder	——	——	——	5.8
Ash of miso	5.0	——	5.0	5.0
$CaCO_3$	1.0	1.0	1.0	1.0
Butter**	7.5	7.5	5.0	7.5
Oryzanin***(c.c.)	5.0	5.0	5.0	5.0

* As dry matter

** Preparation of the Meiji Confectionary Company.

*** Preparation of the Sankyō Pharmaceutical Co.

The protein contents and the calories of the above diets were as follows:

Table III. Protein Contents and Calories of the Diets. (%)

	(I) Control group	(II) Miso group	(III) Kōridōfu group	(IV) Fish powder group
Proteins in polished rice	6.1	5.1	5.5	5.7
Proteins in miso, kōridōfu and fish powder	——	5.4	5.3	5,5
Total protein	6,1	10.5	10.8	11.2
Total-N	0.98	1.67	1.72	1.79
Calories	328.5	345,7	349.3	358.0

As is shown in the above table III the diet of the control group (I) contained only 6.1% protein, while in those of (II), (III) and (IV) groups the protein contents were increased to 10.5, 10.8 and 11.2% respectively by the addition of "miso", "kōridōfu" and fish meat powder. The calorific values were nearly the same, except for those in the control group.

Experiment (B).

The composition of diets in this experiment were essentially the same as those of the previous experiment (A) except that the polished rice which contains no embryo was replaced by that containing the embryo ("haigamai"). For simplicity the latter will be denoted as "polished embryo rice".

Table IV. Protein Contents and Calories of the Diets. (%)

	(I) Control group	(II) Miso group	(III) Kōridōfu group	(IV) Fish powder group
Proteins in polished embryo-rice	6.9	5.8	6.3	6.5
Proteins in miso, kōridōfu and fish powder	——	5.4	5.3	5.5
Total proteins	6.9	11.2	11.6	12.0
Total-N	1.10	1.78	1.84	1.93
Calories	334.5	351.8	354.9	364.3

Young albino rats weighing about 30~40 g. were previously fed for one week with unpolished rice flour, and those which had grown at a normal rate were taken and fed on the above diet. The ingredients were thoroughly mixed with water and cooked in a water bath for about half an hour. During the first 20 days each group consisting of two rats was given daily 15 grams of the diet and after that time 20 grams. The experiment lasted for 68 days from October to December. The growth curves are shown in the following charts:

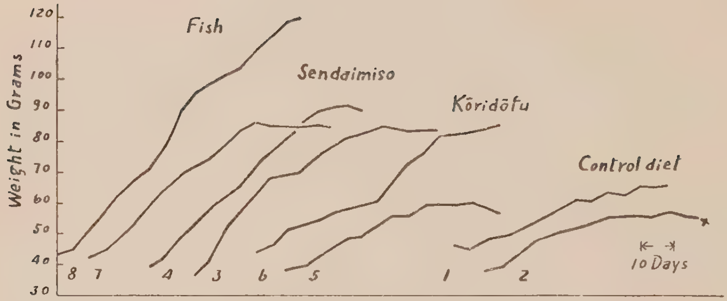

Chart I. Experiment (A). Growth curves of male rats fed on polished rice supplemented with "Sendaimiso", "kōridōfu" and fish powder.

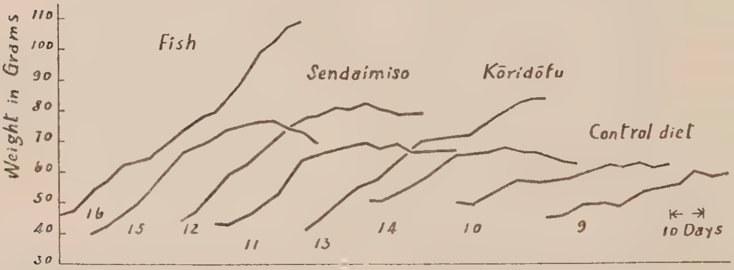

Chart II. Experiment (B). The same fed on polished embryo-rice supplemented with "miso", "kōridōfu" and fish powder.

From the above charts we see that the growth curves are in order of fish powder (IV), "miso" (II), "kōridōfu" (III) and control group (I). The last group attained 60 grams.

II. *Experiment with various kinds of " Miso ".*

The samples used in this experiment were "Shiromiso", "Yedomiso", "Sendaimiso", "Inakamiso" and "Hatchōmiso", "kōridōfu" and fish meat powder were also used for comparison. By the preparation of these kinds of "miso", the raw materials were mixed in the following proportion :

Table V. Raw Materials of "Miso". (By Volume)

Kind of Miso	Soy-bean	Polished rice	Barley	Salt
Shiromiso	1.00	2.00	——	0.30
Yedomiso	1.00	1.00	——	0.40
Sendaimiso	1.00	0.30	——	0.40
Inakamiso	1.00	——	1.00	0.46
Hatchōmiso	1.00	——	——	0.25

The soy-beans were steeped and steamed for several hours according to the kind of "miso" used and while still hot they were thoroughly mixed with rice-"koji" and common salt. For "Inakamiso" barley "kōji" was used instead of rice-"kōji", and for "Hatchōmiso" soy-bean "kōji" alone was used. The mixture was then pressed into a fermenting tank. In "Shiromiso" the ripening was completed in 3∼5 days, in "Yedomiso" in 10∼30 days while in "Inakamiso" and in "Sendaimiso" 11∼12 months were required for complete ripening. The chemical composition of these samples were as follows :

Table VI. Chemical Composition of the Samples used in the Experiment. (%)

Material	Water	Crude protein	Pure protein	Carbohydrates	Crude fat	Ash	NaCl
Shiromiso	52.00	9.31	6.44	31.90	2.70	6.12	4.71
Yedomiso	46.48	12.63	7.31	23.90	5.32	8.25	6.35
Sendaimiso	52.92	13.38	6.13	11.89	3.95	13.96	11.36
Inakamiso	54.60	12.00	5.19	8.61	5.21	13.75	11.71
Hatchōmiso	46.37	21.00	10.94	4.89	6.15	12.88	9.78
Polished rice	7.68	7.46	7.36	79.98	0.81	0.65	——
Polished embryo-rice	7.20	8.35	8.19	78.63	1.30	0.78	——
Kōridōfu	6.45	58.27	58.15	5.20	26.88	4.55	——
Fish powder	2.48	97.81	97.36	——	——	1.13	——
Soy-beans	6.99	35.50	35.28	26.33	19.52	5.01	——
Patrogen(5)	1.24	14.63	——	68.31	10.94	4.68	——

These samples were mixed in the following proportion :

Table VII. Compsition of the Diets.

Material	(I) Shiromiso group	(II) Yedomiso group	(III) Inakamiso group	(IV) Hatchōmiso group
Polished rice	68.7	74.4	74.0	82.7
Shiromiso	27.6*	——	——	——
Yedomiso	——	22.7*	——	——
Inakamiso	——	——	24.8*	——
Hatchōmiso	——	——	——	13.7*
Ash of Sendaimiso	1.8	1.7	——	1.7
Soy-bean oil	0.9	0.2	0.2	0.9
$CaCO_3$	1.0	1.0	1.0	1.0
Butter**	7.5	7.5	7.5	7.5
Oryzanin (c.c.) ***	5.0	5.0	5.0	5.0

Material	(V) Control group	(VI) Sendaimiso group	(VII) Kōridōfu group	(VIII) Fish powder group
Polished rice	91.5	79.3	84.8	86.0
Sendaimiso	——	18.8*	——	——
Kōridōfu	——	——	9.2	——
Fish powder	——	——	——	5.5
Ash of Sendaimiso	5.0	——	5.0	5.0
Soy-bean oil	2.5	0.9	——	2.5
$CaCO_3$	1.0	1.0	1.0	1.0
Butter**	7.5	7.5	7.5	7.5
Oryzanin (c.c.)***	5.0	5.0	5.0	5.0

* As dry matter.
** Preparation of the Meiji Confectionary Co.
*** Preparation of the Sankyō Pharmaceutical Co.

Experimental animals.

Sixty five spring-born rats weighing 30~40 g. (average 35.1 g.) were picked out and fed on "Patrogen"* (a kind of milk powder containing all the dietary factors essential for the growth of children). The average body weight increased from 35.1 to 52.2 g. in 10 days. The growth rates being nearly the same as those of Donaldson, Dunn and Watson(6), as is shown in the charts :

From 65 rats reared as above stated, 36 healthy ones were selected and divided into three groups, each consisting of three rats which were fed on the diets shown in Table VII.

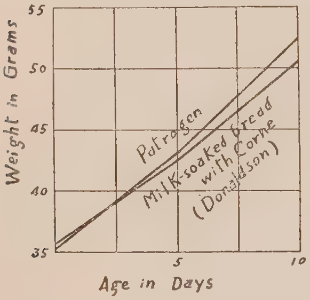

Chart III. Growth curves during preliminary feeding.

Experiment (A).

The growth curves are shown in chart IV and V.

Fig. I shows that the rats fed on "Sendaimiso" grew healthy but those (Fig. II) fed on the "Hatchōmiso" diet were still better as shown in Table VII. Those fed on the "Inakamiso" diet (Fig. III) grew more slowly at first, but continued to grow until the end of the experiment, while the rats fed on the "Shiromiso" diet exhibited a remarkable growth for some time, but during the last forty days no noticeable increase in weight was observed. The animals (Fig. IV) fed on the fish diet grew at an approximately normal rate, while those fed on the "kōridōfu" diet hardly reached 108 or 130 g. in 100 days. This is in accordance with the observation of Suzuki, Okuda and

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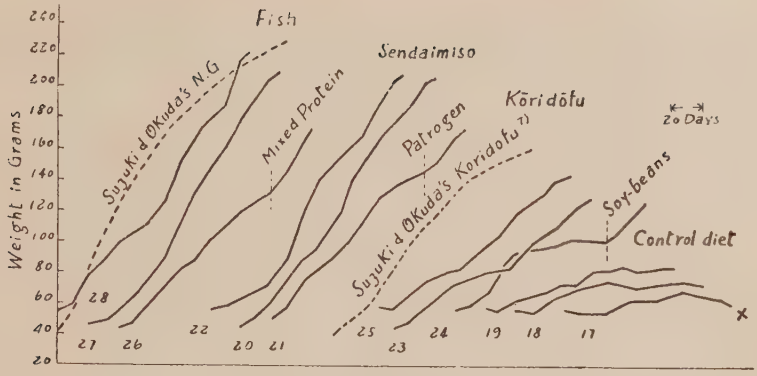
* Preparation of the Meiji Confectionary Company.



× ……indicates death.

Chart IV. Showing growth curves of male rats fed on the polished rice diet supplemented with "Sendaimiso", "kōridōfu" and fish meat powder respectively.

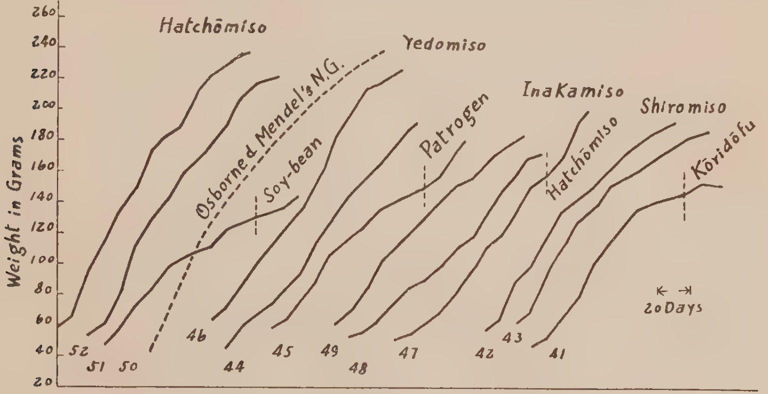


Chart V. Showing growth curves of male rats fed on the polished rice diet supplemented with various kinds of "miso".

Matsuyama(7) who attributed the lower nutritive value of "kōridōfu" to the loss of certain proteins of high nutritive value during the process of making.

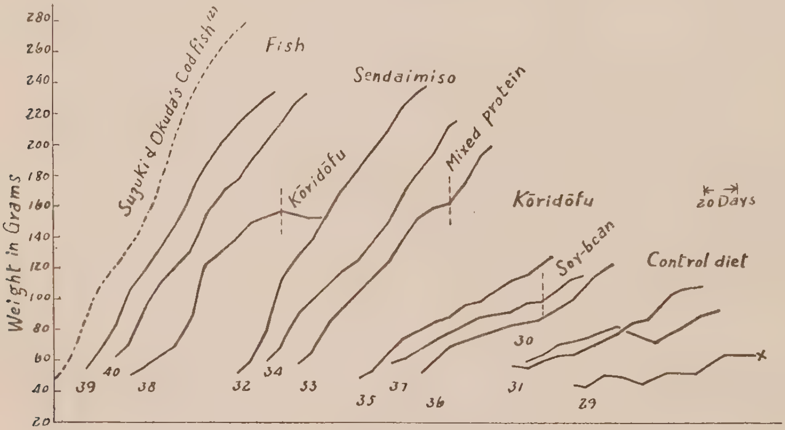
From these results, it can be seen that the greater the amount of soybeans in "miso", the higher is the supplementary value. The fact is in accordance with the observation of Daniels and Nichols(8), Osborne and Mendel(9), McCollum, Simmonds and Parsons(10), and Johns and Finks(11), namely that soybean proteins are capable of supplementing the low nutritive value of cereals.

Experiment (B).

Polished embryo-rice supplemented by "Sendaimiso", "kōridōfu" or fish, produced better growth results than the polished rice diet. The rats (R. 39 and R. 40) fed on the fish diet showed the best development and almost the same appearance as that of rat(12) No. (2), with a diet consisting of 10～18% codfish protein, 43～52% starch, 27～28% protein free milk and 10～

18% butter. Those fed on the "Sendaimiso" diet continued to grow at an approximately normal rate, while those fed on the "kōridōfu" diet gained in weight more slowly. Animals on the control diet containing 7.7% protein of polished embryo-rice have shown very slow growth, one of them gradually declined in weight and finally died, as did the rats on the control diet with polished rice. When rats Nos. 24 and 37 previously fed on the "kōridōfu" diet, were changed to the soy-bean diet at the same level of protein intake, they became more healthy in every respect than the rest of the group. The rats No. 33 on "Sendaimiso" diet, and No. 26 on fish, when placed on a 20% protein intake, 65.5% polished rice, 18.8% "Sendaimiso", 9.2% "kōridōfu" and 5.5% fish, showed an accelerated growth response. The rats No. 21 on the "Sendaimiso" diet and No. 48 on the "Yedomiso" diet, also showed the remarkably good quality of the protein of "Patrogen".

The growth curves are shown in the following chart.



×……indicates death.

Chart VI. Showing growth curves of male rats fed on the polished embryo-rice diet supplemented with "Sendaimiso", "kōridōfu" and fish powder respectively.

The average gain of each group and the food intake in 100 days are as follows:

Table VIII. Rate of Growth. Rats on the polished rice diet.

Kind of diet	No. of rats	Durations of ex-periments	Initial body weigt grams	Gain in 125 days grams	Gain in 100 days grams	Average gain in 100 days grams	Food intake per rat in 100 days grams*
Control diet	17	118	57	——	8	15.7	500
	18	150	58	73	17		
	19	150	58	84	22		

* The daily food intake was only approximately estimated.

Sendamiso diet	20	150	46	218	130	112.0	1140
	21	100	53	——	91		
	22	150	57	212	115		
Kōridōfu diet	23	150	44	130	54	54.7	860
	24	100	58	——	44		
	25	150	59	144	66		
Fish powder diet	26	100	42	——	92	116.5	1080
	27	150	45.5	211	131.5		
	28	150	55	223	126		
Shiromiso diet	41	100	46.5	——	99.5	113.5	1190
	42	150	56	193	127.0		
	43	150	59	187	115		
Yedomiso diet	44	150	42.5	193	120.5	120.5	1160
	45	100	56	——	91		
	46	150	59	227	150		
Inakamiso diet	47	100	49	——	103	99.0	1060
	48	150	50	173	90		
	49	150	59	185	104		
Hatchōmiso diet	50	100	46	——	83	129.7	1250
	51	150	51	222	150		
	52	150	57	236	156		

Rats on the polished-embryo rice diet.

Control diet	29	119	42	——	19	25.3	660
	30	150	56	92	20		
	31	150	56	107	37		
Sendaimiso diet	32	150	49	237	157	129.3	1200
	33	100	54	——	106		
	34	150	58	215	123		
Kōridōfu diet	35	150	46	127	63	51.0	850
	36	150	50	123	48		
	37	100	56	——	42		
Fish powder diet	38	100	48	——	109	135.7	1160
	39	150	51	234	156		
	40	150	56	233	142		

Experiment (C).

In the preceeding experiments (A) and (B), the daily ration during the first 40 days was 10 g. per rat, thereafter it was increased to 15 g. After

125 days when the experiment was finished, most of the animals were used for another experiment. The composition of each diet was the same as before, but the quantity was so adjusted that each rat received exactly 0.2 g. nitrogen per day. This corresponds to 11～12 g. diet according to the kind of "miso" to be tested. After the preceeding investigations, the following 50 days, i.e. from the 126～175 th day (171st～195th day from the birth), were divided into two periods. During the first period (126～150th day) each rat received the same diet as in previous feeding and in the second period (151st ～175th day) the diet was changed, and the average rate of growth in both periods was estimated, for instance:

1. $$\frac{\text{Aver. rate of growth in the second period on "kōridōfu" diet}}{\text{〃 〃 〃 in the first period on "Hatchōmiso" diet}} = 0.6.$$

2. $$\frac{\text{Aver. rate of growth in the second period on "Sendaimiso" diet}}{\text{〃 〃 〃 in the first period on "Yedomiso" diet}} = 0.9.$$

3. $$\frac{\text{Aver. rate of growth in the second period on "Sendaimiso" diet}}{\text{〃 〃 〃 in the first period on "Inakamiso" diet}} = 1.2.$$

4. $$\frac{\text{Aver. rate of growth in the second period on "Hatchōmiso" diet}}{\text{〃 〃 〃 in the first period on "Shiromiso" diet}} = 1.5.$$

5. $$\frac{\text{Aver. rate of growth in the second period on "Yedomiso" diet}}{\text{〃 〃 〃 in the first period on "fish powder diet}} = 0.8.$$

6. $$\frac{\text{Aver. rate of growth in the second period on "Patrogen" diet}}{\text{〃 〃 〃 in the first period on "Sendaimiso" diet}} = 0.9.$$

From Donaldson, Dunn and Watson's data, relating to the growth of male rats of corresponding age fed on the adequate diet (milk soaked bread and corn), the following was calculated.

$$\frac{\text{Aver. rate of growth during 25 days from 196～220th day (from the birth)}}{\text{Aver. rate of growth during 25 days from 171～195th day (from the birth)}}$$

$$= \frac{0.43}{0.53} = 0.8$$

In the present experiment, when one and the same diet was given during both periods, the ratio was found to be about 0.8. Therefore the author assumed that, when the diet in the second period is changed and the ratio becomes larger than 0.8 it indicates that the nutritive value of the second diet is higher than that of the first, and vice versa.

From the experimental data given above, we see that "kōridōfu" and "Shiromiso" are lower in value than "Hatchōmiso". "Inakamiso" is lower than "Sendaimiso" and the latter is equal to or a little better than "Yedomiso" but inferior to "Patrogen", etc.

These results nearly agree with those of the experiments (A) and (B). It should be mentioned here that, the amount of non-protein-N in "miso" is always higher than in "kōridōfu" or in fish powder, nevertheless, the rate of growth, induced by the same quantity of N., i.e. by 0.2 g. N. per day, is higher in "miso" than in "kōridōfu", though a little lower than in fish powder. Thus it may be assumed that the non-protein-N in "miso" is utilized, at least partly, for building up the body protein in rats.

This confirms the observations of S. Maeda(13), that the acid hydrolytic products of proteins when supplemented with tryptophane, can fully replace the proteins in a given diet.

The following analytical data will serve to give some idea of the relation between the constituents and the nutritive value of proteins in various kinds of food articles, though there exists some unknown factor which plays an important role in the nutritive value of proteins.

Table IX. Distribution of Nitrogen in the Food Materials used in the Preceeding Experiments. (%)

	Water	Total–N	Argi-nine–N	Histi-dine–N	Lysine–N	Cystine–N	Trypto phane
Polished rice(14)	7.68	1.19	0.15	0.10	0.06	0.01	0.09
Polished embryo-rice	7.20	1.34	0.24	0.09	0.06	0.01	0.10
Siromiso	52.00	1.49	0.15	0.05	0.09	——	0.11
Yedomiso	49.48	2.02	0.13	0.05	0.08	——	0.12
Sendaimiso	52.92	2.14	0.17	0.06	0.07	——	0.08
Inakamiso	54.60	1.92	0.13	0.05	0.07	——	0.07
Hatchōmiso	46.37	3.36	0.18	0.09	0.14	——	0.05
Kōridōfu	6.45	9.30	1.18	0.30	0.57	0.08	0.85
Fish powder	2.48	15.65	1.93	0.52	1.72	0.11	1.18
Soy-bean(15)	6.99	5.68	0.88	0.15	0.40	0.09	0.51
Rat muscle	2.10	16.21	1.98	0.55	1.76	0.14	1.26

Arginine, histidine and lysine were estimated by Van Slyke's method. Cystine–N by Okuda's method(16) and tryptophane by May & Rose's method improved by Matsuyama & Mori(17).

Summary.

(1) A series of feeding experiments was carried out with white rats to determine the nutritive value of various kinds of "miso", when used as a supplement of polished rice.

(2) The supplementary value of "miso" proteins was found to lie between fish proteins and "kōridōfu", in the order of "Hatchōmiso", "Sendaimiso",

"Yedomiso", "Shiromiso" and "Inakamiso", the last being the lowest.

(3) The nutritive value of "miso" seems to depend chiefly on the amount of soy-beans, used as raw material, and not on the duration of fermentation. The lower value of "Inakamiso" may be explained by the lower content of soybeans.

(5) These experiments have clearly shown that "miso" plays a very important role in the nutrition of the Japanese people, especially in supplying the deficiency of rice proteins.

My best thanks are to Professors U. Suzuki and R. Sasaki for their helpful criticism and encouragement throughout these investigations. I am also greatly indebted to Mr. Sakurai who kindly consented to carry out the chemical analyses and to the Trade Association of the Tōkyō Miso Manufacturers, for kindly supplying the funds and the specimens of soy-bean pastes used in these experiments.

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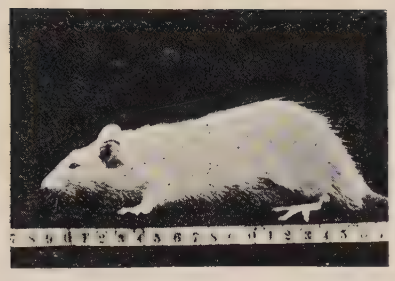

Fig. I Rat No. 20, fed on "Sendaimiso" 176 g. after 100 days.

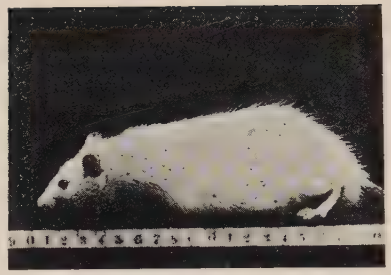

Fig. II Rat No. 52, fed on "Hatchomiso" 213 g. after 100 days.

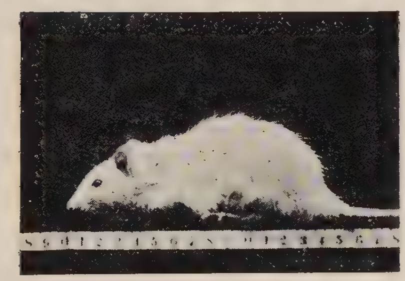

Fig. III Rat No. 49, fed on "Inakamiso" 163 g. after 100 days.

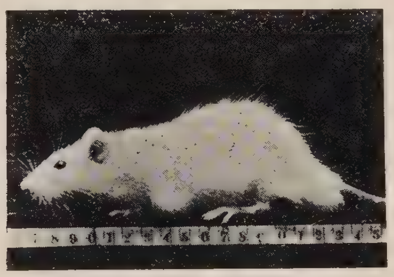

Fig. IV Rat No. 28, fed on fish 181 g. after 100 days.

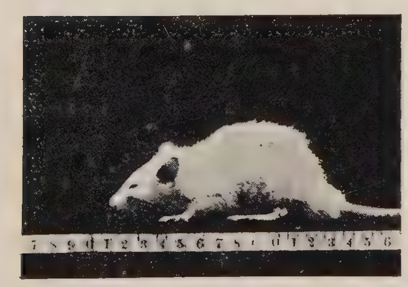

Fig. V Rat No. 25, fed on "kōridōfu" 125 g. after 100 days.

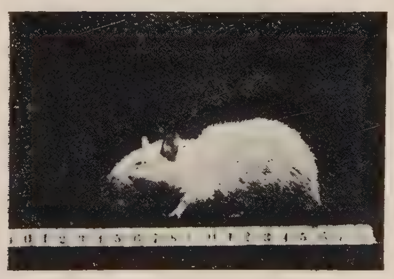

Fig. VI Control rat No. 19, fed on polished rice diet, 80 g. after 100 days.

Investigation on the Influence of Aerial-Earth Circuit on the Biological Activities.

II. Mechanism of the influence on Azotobacter chroococcum as to its potential.

By

Arao ITANO. Ph. D.

(Received August 13, 1933).

As the first report⁽¹⁾ in this series of investigation, it was experimentally proven that the growth as well as the fixation of atmospheric nitrogen of Azotobacter chroococcum was activated in the closed aerial-earth circuit and even by earthing only. Subsequently an enquiry was made as to the mechanism of activation in regard to the potential of Azotobacter chroococcum culture, and the results are reported here as the second report.

Experimental Procedure.

The same strain of Azotobacter chroococcum and the medium were used as noted in the first report, but the flasks were equipped slightly differently so that the potential of the culture is determined easily and all the manipulations can be carried out aseptically, as shown in Fig. I.

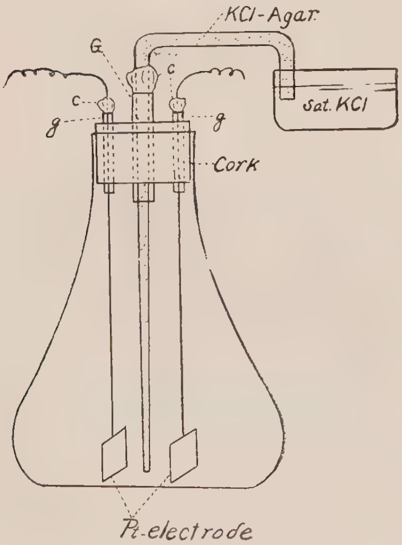

Fig. I.

Each flask was provided with a cork which has two glass tubes, *g* (4 mm. diameter) through either one or both, the platinum electrode is placed and held in position, and plugged with cotton. An another glass tube, *G* (8 mm. diameter) with cotton plug *c* was provided through which a sample for pH determination is taken and also an agar bridge is inserted when the potential is determined. The agar bridge is drawn to capillary at its end and considerably longer than the depth of the flask so that the tip can be broken off at every determination to keep it from contamination. The outer surface of the bridge is sterilized with alcohol and flamed before used. All through the investigation, very careful precautions were taken against contamination and agitation.

The potential of Azotobacter chroococcum culture was determined against the sterile culture medium and also against the saturated calomel clectrode by the following chains I and II respectively, using the K-type potentiometer:

Pt (blank)	Sterile culture medium.	Sat. KCl (agar-bridge.)	Culture	Pt (blank)............(I)
Hg–HgCl	Sat. KCl	Sat. KCl (agar-bridge.)	Culture	Pt (blank)............(II)

The electromotive force thus determined, was converted into Eh, the standard E. M. F., or the normal hydrogen electrode potential. At the same time, the hydrogen electrode potential was determined experimentally, and the difference between that and Eh is given so that the comparable intensity can be inferred.

Influence of Aerial-Earth Circuit on the Sterile Medium.

First it was attempted to ascertain if the potential of the sterile medium is influenced to any extent by the aerial-earth circuit, as follows:

Four Erlenmyer flasks with 100 c.c. sterile Ashby's solution were taken and treated in the following manner: Fask I connected to the antenna and

Table I.—*Influence of Aerial-Earth Circuit on the Sterile Medium.*

Flasks*	Time in hours.	0 (volts)	24 (volts)	48 (volts)	72 (volts)	96 (volts)	120 (volts)
I.	Potential determined	0.8205	0.1454	0.1446	0.1558	0.1574	0.1566
	H_2-electrode potential	0.9355	0.9360	0.9390	0.9440	0.9430	0.9410
	Eh**	0.3675	0.3924	0.3916	0.4028	0.4044	0.4036
	Difference	0.5680	0.5436	0.5474	0.5412	0.5386	0.5374
II.	Potential determined	0.1257	0.1369	0.1383	0.1261	0.1439	0.1250
	H_2-electrode potential	0.9315	0.9400	0.9440	0.9385	0.9400	0.9430
	Eh	0.3737	0.3839	0.3853	0.3731	0.3909	0.3720
	Difference	0.5578	0.5561	0.5587	0.5654	0.5491	0.5710
III.	Potential determined	0.1205	0.1478	0.1556	0.1653	0.1681	0.1589
	H_2-electrode potential	0.9315	0.9410	0.9400	0.9440	0.9385	0.9440
	Eh	0.3675	0.3948	0.4026	0.4123	0 4151	0.4059
	Difference	0.5640	0.5432	0.5374	0.5317	0.5234	0.5381
IV.	Potential determined	0.1262	0.1296	0.1262	0.1280	0.1290	0.1287
	H_2-electrode potential	0.9300	0.9410	0.9400	0.9465	0.9385	0.9205
	Eh	0.3732	0.3766	0.8732	0.3750	0.3760	0.3757
	Difference	0.5568	0.5644	0.5668	0.5715	0.5625	0.5448

Notes: *...Flask I. antenna and earthed; II. earthed only; III. antenna only; IV. no outside connection.

**...Eh, the potential of medium converted into the standard, or the normal hydrogen electrode potential.

earthed; Flask II earthed only; Flask III connected to the antenna only; Flask IV no outside connection. The potential was determined at 24 hours intervals under aseptic conditions. The results are given in Table I.

Table I indicates that the potential of Flask IV which is the control, was not changed to any extent, although in the other flasks, the potential increased slightly.

Influence of Aerial-Earth Circuit on the Potential of Azotobacter chroococcum Culture.

The potential of Azotobacter chroococcum culture against the sterile medium and also against the saturated calomel electrode, was determined as follows:

Four flasks A, B, C and D which were treated same as Flask 1, II, III and IV respectively in the previous case, except all these flasks were inoculated with 1 c.c. of 48 hours old culture of Azotobacter chroococcum. To

Table II.—*Influence of Aerial-Earth Circuit on the Potential of Azotobacter chroococcum Culture.*

Flask*	Time in hours	0 (volts)	24 (volts)	48 (volts)	72 (volts)	96 (volts)	120 (volts)
A.	Potential determined	0.1612	0.0685	0.0906	0.0447	0.0262	0.0453
	H_2-electrode potential	0.9315	0.9220	0.9340	0.9210	0.9205	0.9205
	Eh**	0.4082	0.3155	0.3376	0.2917	0.2732	0.2923
	Difference	0.5233	0.6065	0.5964	0.6293	0.6473	0.6282
B.	Potential determined	0.1717	0.0650	0.0649	0.0331	0.0223	0.0308
	H_2-electode potential	0.9300	0.9140	0.9260	0.9320	0.9320	0.9320
	Eh	0.4187	0.3120	0.3119	0.2801	0.2693	0.2778
	Difference	0.5113	0.6020	0.6141	0.6519	0.6627	0.6542
C.	Potential determined	0.1629	0.0514	0.0411	0.0083	−0.0234	−0.0307
	H_2-electrode potential	0.9335	0.9190	0.9170	0.9140	0.9280	0.9280
	Eh	0.4099	0.2984	0.2881	0.2553	0.2236	0.2163
	Difference	0.5236	0.6206	0.6289	0.6587	0.7044	0.7117
D.	Potential determined	0.1557	0.0496	0.0348	0.0009	−0.0127	−0.0193
	H_2-electrode potential	0.9330	0.9180	0.9150	0.9150	0.9290	0.9280
	Eh	0.4027	0.2966	0.2818	0.2479	0.2343	0.2277
	Difference	0.5303	0.6214	0.6332	0.6671	0.6947	0.7003
D : IV.	Potential determined	0.0100	−0.0610	−0.0882	−0.0937	−0.1160	−0.1588
	Eh	0.2570	0.1860	0.1588	0.1433	0.1310	0.0882

Notes: *...Flasks A, B, C and D were treated same as Flasks I, II, III and IV. in Table I respectively but inoculated.

**...The potential of Flask D determined against Flask IV.

determine the potential of the culture against the sterile medium, Flask D was chained against Flask IV. The results are shown in Table II and Fig. II.

Table II and Fig. II indicate that the potential difference is plainly determined between the culture and the sterile medium. The potential in all the inoculated flasks showed a sudden drop within the first 24 hours, but after that the drop was different by the flask under different treatment. In flasks I, the least decrease of potential took place which was followed by Flask II while in Flask III and IV, the negative potential was observed.

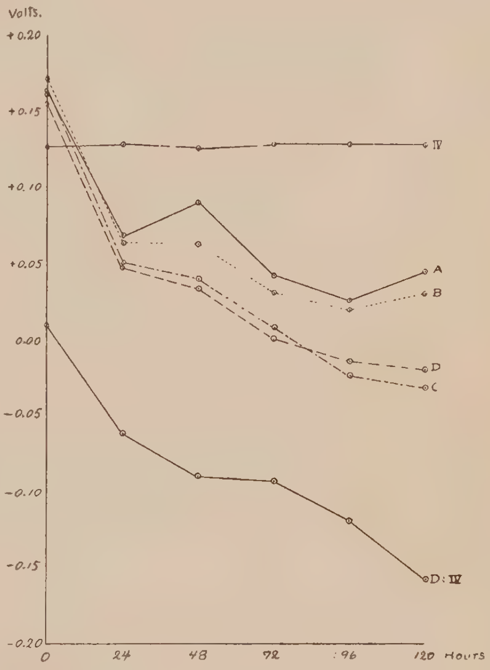

Fig. II—Potential of Azotobacter choococcum under various treatment.

Notes: A—antenna and earthed ; B—earthed only ;
C—antenna only ; D—normal control ;
IV—sterile medium.

Discussions.

So far as the the auther is aware, no previous investigation of this nature on Azotobacter chroococcum has ever been undertaken. Consequently it is very difficult to give a satisfactory interpretation of the results obtained.

As to the nature of potential measured in the bacterial culture, Potter(2) who first measured the potential of Bac. coli in different media and also that of yeast, considered that such potential was electrical. However Gillespie(3) later demonstrated that such potential is a special case of the oxidation-reduction potential indicating the intensity of such reaction. Whatever may be the exact nature of such potential, it is certain that the potential is electrically measurable and such the measurement indicates the intensity of ionic activity in the culture.

In this investigation, it was attempted to ascertain, if such an intensity is influenced by the aerial-earth circuit or not, so that the mechanism of the activation obtained in the previous investigation may be understood.

So far as the data obtained and given in Table II and Fig. II, it is plainly indicated that the different arrangement has different influence on the potential of Azotobacter culture.

Summary.

The results obtained in this investigation indicate that the potential of Azotobacter chroococcum culture is influenced by the aerial-earth circuit.

Considering these results in the light of those which were noted in the first report, the difference of potential which is due to the different treatment, may be a factor bringing about the difference in fixation of nitrogen and growth of Azotobacter chroococcum.

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Ueber die chemischen Bestandteile des Tabaks. III. Mitteilung.

Ueber die Farbstoffe der Tabakblüten. (I).

Von

Kazuo YAMAFUJI.

(Aus dem Biochem. Institut der Landw. Abteilung. der Kaiserl. Kyūshū-Univ. zu Fukuoka, Japan.)

(Eingegangen am 20, Juli 1933).

Trotz der zahlreichen Untersuchungen über die Bestandteile des Tabaks ist noch wenig über die der Blüten desselben bekannt. In der zweiten Mitteilung(1) hat der Verfasser die Isolierung eines Flavonglucosids, welches er Tabacinin genannt hat, aus den Tabakblättern beschrieben. In der vorliegenden Arbeit möchte er über seine Forschungen über die Farbstoffe der Tabakblüten berichten.

Nach meiner Erfahrung enthalten die Blüten des Tabaks, der früher eine Zierpflanze war, ausser einem Anthocyan noch ein Flavon, um deshalb die phytobiochemische Beziehung zwischen diesen beiden Farbstoffen zu studieren, versuchte der Verfasser die Isolierung dieser Farbstoffe aus den Tabakblüten. Verfasser teilt hier wegen der geringen Ausbeute an Farbstoffen und ferner der Schwierigkeit der Sammlung der Blüten wegen seine noch unvollendeten Untersuchungen nur vorläufig mit.

A. Ueber das Anthocyan.

Die Blüten des Tabaks sind sehr arm an Anthocyan. 63000 von den Kelchen befreite Blüten lieferten 1260 g. trockenes Blütenmehl, woraus nur 0.6 g. Anthocyan-Pikrat isoliert wurden. Die Extraktion des Farbstoffs erforgte mittels methylalkoholischer Salzsäure. Zur Reinigung wurde zuerst das Anthocyan in Bleisalz und dann in Pikrat verwandelt. Der geringe Ausbeute wegen musste der Verfasser auf Krystallisationsversuche verzichten. Die Hydrolysenversuche erwiesen aber dass das isolierte Anthocyan schon genügend rein ist; es besteht aus 1 Mol. Monosaccharid und 1 Mol. Anthocyanidin. In späteren Untersuchungen will ich die Identifizierung des Zuckers und freien Farbstoffs ausführen.

Experimentelles.

(1) Isolierung: — Die frisch gepflückten Tabakblüten wurden von den Kelchen befreit und im Trockenschrank bei ca. 60° getrocknet. 1.2 kg. trocknetes Blütenmehl wurden mit 212 proz. methylakoholischer Salzsäure übergossen, über Nacht stehen gelassen, hierauf abgenutscht und mit 1 proz. methylalkoholischer Salzsäure wiederholt nachgewaschen. Aus dem Extrakt werde der rohe Farbstoff durch Zusatz des dreifachen Volumens Aether gefällt. Diesen Niederschlag löste man in 1l warmem Wasser und fügte dazu die gesättigte Bleiazetatlösung. Der Bleiniederschlag wurde mit Methylalkohol und dann mit Aether gewaschen und hierauf mit 7 proz. methylalkoholischer Salzsäure geschüttelt. Nach der Abtrennung des Bleichlorids wurde das Anthocyan als Chlorid aus dem Filtrat mit Aether niedergeschlagen. Die Fällung nahm man in einer kleinen Menge warmem wasser auf und brachte durch Zusatz von heiss gesättigter Pikrinsäurelösung das Anthocyan-Pikrat zur Ansfällung. Das Pikrat wurde nochmals in 5 proz. methylalkoholischen Salzsäure gelöst und das Farbstoffchlorid erneut durch Aether gefällt. Dieses Chlorid, nach dem Auflösen in warmem Wasser, wurde durch Pikrinsäure wieder in Pikrat verwandelt.

(2) Eigenschaften: — In Wasser oder Methylalkohol löst sich das Anthocyanchlorid mit purpurroter Farbe leicht, aber nicht in Aethyl- oder Amylalkohol. Eisenchlorid ruft in der Lösung des Farbstoffes eine schwach grüne Färbung hervor; Natriumcarbonat gibt eine Violettblau, Bleiazetat eine violettrote Fällung.

(3) Hydrolyse: — 0.2077 g. des bei 100° im Vakuum getrockneten Farbstoff-Pikrats wurden mit 15 ccm 20 proz. Salzsäure 3 minuten gekocht. Nach dem Erkalten wurden das auskrystallisierte, zuckerfreie Farbstoffchlorid abfiltriert und mit etwas 20 proz. Salzsäure nachgewaschen. Die salzsaure Mutterlauge wurde zur Entfernung der gelösten Pikrinsäure ausgeäthert und hierauf das in der Lösung zurückbliebene Anthocyanidin mit Amylalkohol

ausgezogen. Aus den amylalkoholischen Extrakten wurde das Lösungsmittel verdampft, der hinterbliebene Farbstoffrest in Methylalkohol gelöst und mit Aether gefällt.

Die wässerige saure Flüssigkeit, nach Entfernung des gelösten Amylalkohols durch Ausschütteln mittels Aether, wurde mit Natriumbicarbonat neutralisiert, mit Knochenasche entfärbt, filtriert, auf 50 ccm. gebracht und zur polarimetrischen Bestimmung des Zuckers benützt.

In der sauren Spaltung des Pikrats (0.2077 g.) isolierte, bzw. bestimmte ich;

Pikrinsäure……0.0403 g., Anthocyanidin-chlorid……0.0965 g.,
Zucker (als Glucose)……0.0429 g.

Eine zweite Hydrolyse führte zu nachfolgenden Werten;

Aus 0.0926 g. des Farbstoff-chlorides.
Anthocyanidin-chlorid… · 0.0595 g., Zucker (als Glucose) ……0.0228 g.

B. Ueber das Flavon.

Das Tabakblütenmehl, nach Extraktion des Anthocyans, diente zur Isolierung des Flavons. Der Verfasser konnte leider ein reines Flavonglucosid noch nicht herstellen, aber durch die Hydrolyse eines Niederschlags, welcher zweifellos das Flavonglucosid enthält, eine sehr geringe Menge von dem freien, krystallisierten Flavon erhalten. Ueber den Zucker und das Flavon, die durch die saure Spaltung des rohen Glucosids erhalten wurden, will der Verfasser seine Untersuchungen noch fortsetzen.

Experimentelles.

(1) Isolierung: — Das Blütenmehl des Tabaks, nach Entfernung des Anthocyans mittels methylalkoholischer Salzsäure, wurde mit Calciumcarbonat versetzt und mit 95 proz. Alkohol wiederholt extrahiert. Der alkoholische Auszug wurde mit Natriumhydroxyd neutralisiert, im Vakuum eingedampft, der Rückstand mit warmen Wasser behandelt und filtriert. Aus der wässerigen Lösung erhält man ein gelbe Fällung durch Zusatz von Bleiazetat. Dieser Niederschlag wurde mit verdünnter Salzsäure zersetzt, abgenutscht und stark eingeengt. Der Rückstand wurde zuerst ausgeäthert nnd dann mit Alkohol extrahiert. Die alkoholische Lösung wurde wieder eingeengt und mit Aether gefällt. Ausbeute 1.5 g.

(2) Eigenschaften: — Dieses rohe Glucosid ist hellgelb, und reduziert Fehlingsche Lösung. In der Lösung dieses Glucosides gibt Ferrichlorid eine grüne Färbung, Bleiazetat eine gelbe Fällung, Magnesium und Salzsäure ein schönes Rot. Wenn es in konzentrierter Schwefelsäure gelöst wird, so erhält man eine gelbe Lösung mit einer blaugrünen Fluoreszenz.

(3) Hydrolyse : — 1 g. Glucoside wurde mit 100 ccm. 5 proz. schwefelsäure 6 Stunden erhitzt. Die saure Flüssigkeit, nach dem Erkalten, wurde mit Aether geschüttelt und die ätherische Lösung eingedampft. Wird der Rückstand aus verdünntem Alkohol umkrystallisiert, so erhält man gelbe, nadelförmige Krystalle von freiem Flavon. Die schwefelsaure, wässerig Lösung enthält einen Zucker, dessen Identifizierung noch nicht ausgeführt worden ist.

Zum Schluss danke ich herzlich dem Herrn Prof. Y. Okuda für seine Anregung bei dieser Arbeit.

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Sulphates and chlorides as fertilizers (III.)

Viscosity and surface tension of rice paste.

By

Isenosuke Onodera.

(Received August 3, 1933)

(1) Samples of paddy rice were obtained from culture cylinder in open and paddy fields, the latter of which was devided into small plots by low dykes.

(2) The viscosity of both the polished and unpolished rice increases with the quantites applied, but inverse is true with their surface tension.

(3) The viscosity of the unpolished rice is larger and its surface tension smaller, than those of the polished. This is due to the separating-off of rice bran, containing a large quantity of fat and electrolyte, which have much to do with the viscosity and surface tension.

(4) Relations between the viscosity of the unpolished rice paste and the fertilizer applied were as follows :

(a) The composition of fertilizer, giving the largest viscosity.

Name of experiment		"Uwadai" soil	"Shitadai" soil
Cylinder culture		Ammonium chloride Natrium phosphate Potassium sulphate	Ammonium Sulphate Ca-Superphosphate Potassium Chloride
Field experiment	Mineral fertilizer only	Ammonium Carbonate Ca-Superphosphate Potassium Carbonate	——
	Mineral fertilizer and organic manure	Farm yard manure Soy bean cake Ammonium Chloride Ca-superphosphate Potassium sulphate	——

(b) Relations between viscosity and the compounds of potassium, ammonium so far as their chlorides and sulphates are concerned, were :

The viscosity of the plots of compounds of the same anion are always smaller than those of different anion given, and was no different tendency noticed between the "Uwadai" and the "Shitadai" soils.

(5) Relation between the surface tension of the unpolished rice paste and fertilizer applied were :

(a) The composition of fertilizers, giving the largest surface tension.

Name of experiment		"Uwadai" soil	"Shitadai" soil
Cylinder Culture		Farm yard manure Ammonium chlorides Ca-superphosphate Potassium sulphate	Farm yard manure Ammonium chloride Ca-superphosphate Potassium sulphate
Field experiment	Mineral fertilizer only	Ammonium carbonate Ca-superphosphate Potassium chloride	——
	Mineral fertilizer and organic manure	Farm yard manure Ammonium chloride Ca-superphosphate Potassium chloride Powdered limestone	——

(b) Relations between the surface tension and the compounds of potassium and ammonium, so far as their chlorides and sulphates are concerned, were :

The surface tension of the plots of the compounds of the same anion are generally larger than those of different anion given.

昭和八年九月七日印刷
昭和八年九月十日發行

東京帝國大學農學部內
發行兼編輯者　後藤格次

東京帝國大學農學部內、日本農藝化學會
印刷者　稻垣秀實

東京帝國大學農學部內
印刷所　日本農藝化學會印刷部